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REACTIONS OF THE HONEYBEE TO LIGHT[1]

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I. EXTENT OF THE SPECTRUM FOR THE HONEYBEE AND THE DISTRIBUTION OF ITS STIMULATIVE EFFICIENCY

INTRODUCTION

In considering the reactions of a given animal to light of different wave lengths there are three fundamental questions which perhaps come first to mind: (1) The extent of the spectrum for this animal—that is, the wave lengths of the shortest and longest radiations to which it responds, (2) the relative efficiency of the different wave lengths in eliciting the same response, and (3) the extent to which the animal distinguishes the different wave lengths, or the number of chromas that it distinguishes.

The last of these questions is discussed in Part II of this paper, but the first two may properly be considered now. Before asking these questions in regard to the honeybee in particular, however, it may be well, for comparison, to review briefly our knowledge of the spectra for other animals, so far as investigation has revealed. The results of the more important investigations follow: The spectrum for man (*12*) [3] is said to extend from about wave length 400 mμ at the violet end to about 770 mμ at the red end,[4] the chicken from 500 to 700 (*19*), the pigeon from 424 to 704 (*26*), the alligator from 398 to 704 (*26*), the fish Phoxinus from about 340 to about 800 (*37*), the blowfly larva from about 422 to 625 (*30*), the water-flea Daphnia as low as 228 (*3*), the cyprid larva of the barnacle Balanus from 355 to 690 (*36*), the clam *Mya arenaria* from about 420 to about 650 (*14*), the larva of the worm Arenicola from 442 to 534 (*30*), the earthworm from 463 to 514 (*30*), Cerianthus from 460 to about 653 (*31*), Volvox from 434 to 655 (*27*), and in an investigation of 12 protozoa (*30*) the spectrum

[1] Received for publication Oct. 27, 1930; issued April, 1931. Parts I and II of this paper were presented as a dissertation in partial fulfillment of the requirements for the degree of doctor of philosophy, received in June, 1928, from the Johns Hopkins University, where the writer was a Bruce Fellow. The work upon which they were based, as well as that of Part III, was done while the writer was in the status, first, of junior biologist in apiculture and, later, of field assistant, division of bee culture, Bureau of Entomology, U. S. Department of Agriculture. The experimental work of the first two parts was done largely in the laboratory of the division of bee culture, and in part at the zoological laboratory of the Johns Hopkins University. That of Part III, and the final preparation of the manuscript, were done at the bee culture laboratory.

[2] Now professor of biology, Western Maryland College, Westminster, Md. The writer is indebted to S. O. Mast, of the Johns Hopkins University, for suggesting the problems here considered and giving general supervision to the work on Parts I and II; to James I. Hambleton, in charge of the division of bee culture, Bureau of Entomology, for making possible the investigations and furnishing laboratory facilities; to A. L. Colton, of the Bureau of Entomology, for helpful suggestions on statistical and mathematical questions; to W. W. Coblentz, of the Bureau of Standards, U. S. Department of Commerce, for determinations of spectral energy; and to all here named for much kindly advice.

[3] Reference is made by number (italic) to Literature Cited, p. 417.

[4] A high limit of 850 has been reported (*34, p. 105*) and a low limit of 210 (*29*). The writer can see ultraviolet as low as wave length 365 mμ when his eyes are fully adapted to darkness.

was found to extend from between 422 and 483 at the violet end to between 504 and 646 at the red end.

A word of caution against taking these and similar data too literally is perhaps advisable here. It must be remembered that the difficulties involved in such studies as those cited are very great. In the first place, it is difficult to compare man with other animals, since with man the subject can speak of his subjective impressions, but with other animals one has to observe some physical response as a proof that the light is having an effect. If the light has an effect which is subminimal to the response which one is observing there is no way of ascertaining that fact. In the second place, owing to the difficulty of controlling accurately the wave length of the light used, it is not easy to determine exactly the limits of the spectrum for an animal. The various light filters often employed, although transmitting much of the incident light, transmit at the same time a wide band of wave lengths, with different proportions of each. On the other hand, the sections of a pure prismatic spectrum such as is sometimes employed, although much more homogeneous in wave length, are often too weak to elicit a response at a particular wave length. In the third place, it is often true that an animal still responds at the shortest or even the longest wave length producible by the apparatus used. In such an instance one obviously can not be sure whether he has reached the animal's limit of vision. And even in case the limit has been reached at a given intensity of illumination, there is no reason to suppose that this is an absolute limit, and would not extend farther if the intensity were increased.

Notwithstanding these and other considerations which make it impossible to state precisely and unconditionally what the extent of the spectrum is for a particular animal, certain conclusions may be drawn. It is apparent that the average human eye under usual conditions is sensitive to radiations ranging in wave length between about 400 and 770 mμ. The range for man extends on the red end of the spectrum farther than the range for most of the other animals. Several of the experimenters remark on the fact that red light to which they themselves are sensitive does not seem to stimulate the animals in question. However, in birds, and possibly also in fishes, the opposite seems to hold. Honigmann (*19*) says that for chickens the region around wave length 660 mμ is four times as stimulative as for man, and the fish *Phoxinus laevis* has been credited with being sensitive to radiations of wave lengths nearly or quite as long as 800 mμ (*37*). On the other hand, it is fairly common to find animals sensitive to the shorter wave lengths in the deep blue and violet, e. g., at 425 mμ. Some are said to exceed man in this respect, the fish just mentioned being sensitive to radiations at 340 mμ and Daphnia even to those at 228 mμ.

But what of the honeybee in particular? There has been no direct research on this problem with honeybees, but there is considerable evidence to be derived from the so-called training experiments of Von Frisch (*7*) and Kühn (*22*). These men first trained the bees to associate a given color with food, and then observed whether or not the bees confused that color with other colors or with various shades of gray. Von Frisch, for example, found that after training bees to come for food placed exclusively on red paper, and then after shifting the position of the red in relation to that of various gray papers presented at

the same time, the bees confused dark gray and black with red, although they did not confuse yellow, or blue, or violet, with any gray. This result indicates that the paper which appears red to us appears very dark, almost black to bees; in other words, that their spectrum does not extend as far into the red as our own.

Kühn (*22*), using spectral colors instead of colored papers, got results essentially similar to those of Von Frisch in regard to red. More precisely, he maintains that the bee does not respond to wave lengths longer than 650 mμ. In addition, however, he found that his bees were stimulated by regions of the ultra-violet as low as that of wave length 313 mμ, ordinarily invisible to man. And not only were they stimulated, but after being trained to associate the wave lengths 313 mμ and 365 mμ with the presence of food, they did not confuse these two regions with any other region of the spectrum which he tried—a result which indicates that they see this ultra-violet light as a color distinct from all colors visible to us, and not merely as a fluorescence, and that, consequently, the spectrum visible to them extends at least as far into the ultra-violet as the wave length 313 mμ.

In regard to the honeybee, nothing at all has been known as to what region of the spectrum is most efficient in stimulating it. If it is true that the spectrum visible to the bee extends from wave length 313 mμ in the ultra-violet up to 650 mμ in the orange-red, we should expect to find the maximum point, judging from the usual results, somewhere more than halfway toward the ultra-violet end of the spectrum, probably in the blue. Or perhaps there are two maxima, one in the usual green or greenish-yellow region and another in the violet or ultra-violet. To determine the relative stimulative efficiency of the various parts of the spectrum to which the bee reacts, and to ascertain more carefully its limits, thus become especially alluring problems. The experiments to be described were designed to throw some light on them.

In regard to the second question mentioned at the beginning of this introduction—namely, the relative stimulative efficiency of different wave lengths—comparatively little is known except with respect to the human eye. For man a summary of the various investigations (*12*) places the point of maximum efficiency between the wave lengths 550 and 560 mμ. This standard curve is reproduced in Figure 4 (dotted curve). For other animals the most reliable investigations give the following results: For the chicken (*19, p. 68*) the maximum efficiency is between 560 and 580 mμ, for the pigeon (*26*) it is at 564, for the alligator (*26*) it is at 544, for the fish Phoxinus (*37*) it is probably at 592,[5] for the blowfly larva (*30*) at 504, for the cyprid larva of Balanus (*36*) at about 540, for *Mya arenaria* (*14*) at about 500, for the Arenicola larva and the earthworm (*30*) at 483, for Cerianthus (*31*) at about 532, for Volvox (*27*) at about 494, and for various protozoa (*30*) it ranges between 534 and 473, the location depending on the animal. Taken as a whole, the region of the spectrum most efficient in stimulating animals, so far as these data show, is somewhere between the blue-green and the yellow region; in other words, somewhere between the wave lengths 473 and 580 mμ, according to the particular animal. Only in birds and possibly in fishes does the maximum lie farther toward the red end of the spectrum than in man; in all others it lies

[5] This is the region which the fish most readily learns to associate with food; it may not be the region of actual maximum efficiency.

farther toward the violet end. Where complete curves of the distribution of stimulative efficiency in the spectrum have been worked out they are similar to the curve for man presented in Figure 4. In the curve for any given animal the maximum is nearly always not exactly in the center of the curve, but lies slightly skewed toward the violet end.

METHODS AND RESULTS

One of the most successful methods of finding the relative stimulative efficiency of various wave lengths which are unequal in energy is the one used by Mast in 1917 (*30*). The procedure is, briefly, as follows: The animals are exposed simultaneously to a spectral color and to white light of a measurably variable intensity. This intensity is then varied until the animals react equally to both beams. Then another spectral color is chosen and again the white is equated to it, and so on for as many as may be desired, or as is possible with the apparatus at hand. The relative intensities of the white then represent the relative stimulation caused by the various spectral colors. It should be emphasized that the white is adjusted until its effect on the animal is *equal* to that of each spectral color used; this is a point that will be referred to later. Having found the relative *effect* of each spectral color, the relative *efficiency* of each can then be calculated by simply dividing the relative effect by the relative energy transmitted at that wave length.

This method, in essentials, was used in the experiments here described, although several modifications had to be made, which will be considered at the proper place.

At the bee culture laboratory there was available a spectroscope of the constant-deviation type, with which to obtain light of the wave lengths desired. A 400-watt gas-filled projection lamp having a tungsten filament furnished the light for the spectrum. The width of the collimator slit of the spectroscope was made 1.8 mm. and that of the telescope slit 1.05 mm. The graduated head of the instrument, directly denoting the wave length of the light used, read from 430 mμ to 700 mμ. The readings for these experiments were chosen at intervals of 20 mμ from 430 mμ, giving 13 steps up to and including 690 mμ, or, in all, 14 wave lengths. On subsequent calibration of the instrument with a helium tube, the indicated wave length 430 mμ was found to be really 431 mμ, and 690 mμ to be really 697.5 mμ, with intermediate points proportionately in error; the corrected values are therefore substituted for those indicated by the instrument. It is recognized, of course, that a slit 1.05 mm. in width will not give monochromatic light. The length of spectrum able to pass a telescope slit of given width is, in a prismatic spectrum, greater for the longer waves than for the shorter waves, owing to the greater refraction of the latter. Thus, in this apparatus the light said to have a wave length of 431 mμ was in reality the aggregate of a band extending approximately from 429 mμ to 433 mμ, and similarly that called 690 mμ was a band extending approximately from 675 mμ to 705 mμ, those between varying proportionately.

The first problem was the arrangement of an apparatus in which the bees would react primarily to light and not to other stimuli. For example, one of the most disturbing factors in such work as this is the tendency of bees in close confinement simply to race continuously around the sides of the container, apparently in a frantic effort to escape, and to disregard the light almost entirely. It was

found that to remedy this difficulty the container must be made so large that even with its great activity the bee will seldom strike the sides, but will be able to roam in comparative freedom over a large space. Every effort to confine the bee to narrow paths or make it go through small openings resulted in almost complete substitution of contact reactions for light reactions. After a number of tests the need was satisfactorily met. A shallow, rectangular box, 65 by 25 by 4 cm., was made and covered with a wire screen. Through a glass-covered opening in the middle of one end of this box a narrow beam of light from an ordinary electric bulb could be projected, and through two openings in the opposite end, each closed with a diffusive screen of ground glass, the light from the spectroscope and white light from a comparison lamp could be projected simultaneously, the two screens being of the same shape and size. Each screen was at the end of a tunnel, the two tunnels extending across the

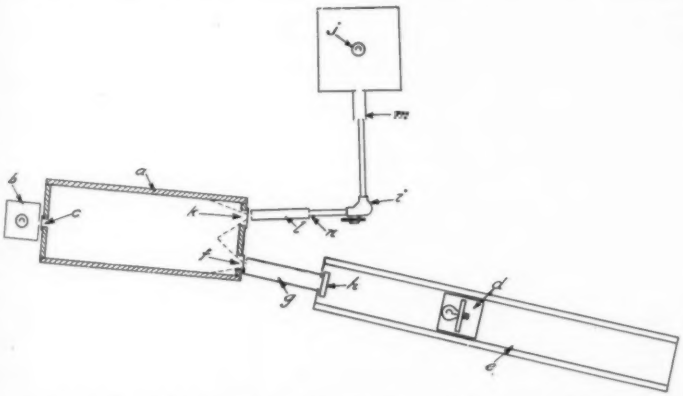

FIGURE 1.—Arrangement of apparatus used to ascertain the relative stimulative effect of spectral rays upon bees; *a*, box in which the bees were inclosed for experimentation; *b*, box containing electric bulb to illuminate ground glass at aperture *c*; *d*, comparison lamp, inclosed in box *e*, and illuminating ground glass at aperture *f*, through tube *g*; *h*, cell containing solution of cupric sulphate; *i*, spectroscope, illuminated by electric lamp *j*, and directing beam of colored light on ground glass at aperture *k* through tube *l*; *m*, collimator slit; *n*, telescope slit

width of the box. The apparatus was, of course, set up in the dark room.

The diagram, Figure 1, is inserted to convey a better idea of the arrangement of the apparatus. The box *a* is 65 by 25 by 4 cm. in size, and covered with a wire screen; in this the bees were confined; *b*, a box, 15 by 15 by 15 cm., in which was placed a 15-watt electric bulb, to illuminate a piece of ground glass which covers the aperture *c* connecting *a* and *b*; at the end of *a*, opposite this aperture, are two passages, with flaring sides, leading to the two apertures *f* and *k*, each covered with ground glass. The ground glass at *f* was illuminated by the 100-watt "comparison lamp" *d*, which was inclosed in a box, *e*, 117 cm. long and provided with guides for conveniently adjusting the distance of the lamp from the ground glass. The only light escaping from this box was emitted through an opening in one end of it, 26 cm. from the ground glass, this opening and the aperture *f* being connected by a tube *g* to prevent

diffusion of the light. At the end of this box, covering the opening, was a glass vessel, *h*, containing a dilute solution of cupric sulphate, to eliminate infra-red light. The glass of this vessel and of the diffusion screens also eliminated ultra-violet. The aperture *k* was connected by a tube *l* with the slit through which the light of the wave length chosen was emitted from the spectroscope.

As has been stated, the spectroscope *i* is of the constant-deviation type, with a graduated head for controlling the wave length of light to be emitted by the slit *n* and thrown on the ground glass at the aperture *k*. The collimator slit *m* was illuminated by a 400-watt electric lamp, *j*, inclosed to prevent the escape of light and placed at a suitable distance.

As a beginning of the routine of experimentation, 10 bees were caught at a feeder or on flowers[6] and were put into the box. A feeder, consisting of a bottle of sugar water, its mouth covered with cloth, was then inverted on the wire screen and the light of the dark room turned off. The bees soon collected on the cloth and began to feed. They were left in darkness for from three to five minutes; in this time they became satiated with sugar water, and quieter and less excitable. They also became somewhat adapted to the darkness. The small lamp (in box *b*, fig. 1) illuminating the opening at the center of one end of the box was then turned on and left until the bees congregated at this end, then turned off, and simultaneously the spectroscope lamp and the comparison lamp were turned on, each producing an illuminated area of the same size on the ground-glass screen used with it at the opposite end of the box. Most of the bees then went toward these areas, part entering one of the two flaring tunnels (fig. 1, *f* and *k*) and part the other, sometimes a few entering neither, but wandering aimlessly, without direction. After the bees entering each tunnel had been counted, the lamp at the opposite end was again turned on and the opening there illuminated until the bees congregated at that end (fig. 1, *c*), then the whole process was repeated. The 10 bees were thus induced to react over and over again until they began to appear sluggish and somewhat indifferent to the light. Ordinarily about 150 positive reactions were obtained from 10 bees. As soon as the bees became unfit for further use they were removed from the box, taken into the open, and released. Then 10 more field bees were captured and put into the box, and 150 positive reactions again obtained, and this procedure was continued as long as desired.

In the earlier experiments an attempt was made so to adjust, by changing the distance of the lamp, the intensity of the beam of white light that the response induced by it equaled that induced by the beam from the spectroscope, but this was found to be almost an endless task. After spending a whole day in counting reactions with the comparison lamp set at a given distance, it was usually found that the response induced by it was not equal to that induced by the given spectral color; so, after slightly increasing or decreasing the distance, as the results demanded, the experiment had to be continued for another day to see if the two beams were now equal; if they were not, the distance had again to be changed and another day spent, and so

[6] Field bees must be used. Young bees that have not yet become field bees are practically indifferent to light.

on. One was never quite sure when the true value had been obtained—never quite sure when to stop.

To avoid this difficulty the method was modified. Instead of varying the intensity of the white light on the ground glass in an effort to make its effect equal to that of the spectral light, the comparison lamp was left at a constant distance and its effect on the reactions of the bees was compared with the corresponding effect of each spectral color. The graduated head of the spectroscope was turned to indicate a given wave length—e. g., 698 mμ—and, after the bees had been induced to assemble at the opposite end of the box, the beam from the comparison lamp and light of the wave length 698 mμ were thrown on the two screens simultaneously. The bees entering each of the tunnels were counted; then the bees were brought back to the starting point again and the process repeated until a total of 25 bees had entered the two tunnels. Then the graduated head was set for another wave length and the procedure repeated, and so on until each of the whole series of 14 different spectral colors had been compared with the constant white. Then the series was repeated, using the 14 spectral colors again, and so on for 20 series—i. e., until the total number of entrances (into both tunnels) observed in the comparison of each spectral color with the white amounted to 500.

For each wave length, in each series, a total of 25 bees, as has been said, entered the two tunnels. Only those entering the tunnel leading to the ground glass illuminated by the spectral color were recorded. To find the relative stimulative effect of light of a given wave length as compared with the stimulative effect of the white light used for comparison, the total number of bees recorded for a given wave length was divided by the corresponding total of those attracted to the white light; in other words, by the difference between 500 and the total recorded. For instance, in the 20 series, a total of 70 bees were attracted to the ground glass by light of wave length 431 mμ. By dividing 70 by 430 (i. e., 500−70) the ratio 0.16 is derived as the relative stimulative effect of light of this wave length as compared with the white light taken as a standard. The results obtained are presented in Table 1. They indicate that, beginning with the shortest wave length and proceeding to the longest, the stimulative effect gradually increases to a maximum at 553 mμ and then decreases.

But a serious question arises here: Do the ratios obtained in this way represent the true relative stimulative effect of the respective wave lengths? For example, if the ratio for one wave length is twice that for another, is the stimulative effect of the former really twice that of the latter? At first thought the answer appears to be in the affirmative, but further consideration shows that these values may *not* truly represent the relative stimulative effect of the various wave lengths. For, according to the method used by Mast (*30*), already described, the true relative stimulative effects of diverse wave lengths are the relative intensities of white required to produce responses equal in magnitude to those produced by the several wave lengths used.

TABLE 1.—*Record of the number of bees attracted by light of selected wave lengths, and ratios of the totals for each wave length to the corresponding totals for the white light which was taken as the standard of comparison*

Series	Number of bees attracted to light of wave length specified (in millimicrons)													
	431	451	471	492	512	532	553	574	595	615	636	657	677	698
1	3	6	6	8	11	10	11	15	11	7	4	4	3	1
2	2	7	6	9	10	8	12	8	6	9	3	4	6	1
3	6	4	7	6	14	10	11	8	9	6	8	4	3	1
4	7	1	6	9	10	13	13	14	8	6	4	5	2	5
5	0	5	9	8	8	12	13	14	9	10	7	4	6	3
6	2	4	5	10	8	15	11	14	14	9	7	2	0	2
7	5	4	8	7	12	18	16	16	9	10	6	4	4	0
8	4	4	9	8	10	15	14	13	15	6	5	5	2	0
9	6	9	8	10	8	10	14	11	14	9	8	6	3	2
10	5	6	9	10	15	13	14	13	12	13	7	3	2	0
11	4	5	10	11	13	10	12	12	8	9	6	4	3	3
12	3	4	7	12	7	10	13	13	11	8	7	6	5	0
13	6	7	8	11	10	10	14	13	9	9	7	3	2	1
14	5	3	9	8	11	11	12	8	14	8	5	5	4	2
15	1	3	6	8	10	9	13	9	11	9	7	4	5	6
16	0	6	7	10	13	13	12	9	8	5	4	5	3	2
17	2	7	4	11	14	13	11	11	11	10	4	5	3	4
18	4	5	5	9	9	6	10	10	13	13	3	3	2	2
19	4	6	7	11	8	12	13	13	10	6	5	1	4	1
20	1	3	6	6	10	10	11	14	11	9	4	3	3	3
Total	70	99	142	182	211	228	250	238	213	171	111	80	65	39
Total attracted to white light	430	401	358	318	289	272	250	262	287	329	389	420	435	461
Ratio	0.16	0.25	0.40	0.57	0.73	0.84	1.00	0.91	0.74	0.52	0.29	0.19	0.15	0.085

Consequently, the question that now arises is this: If a given wave length induces, for example, 25 per cent as great a response as a given white, how much will the white have to be reduced to bring about a response equal to that of the given wave length? Or, we may state the question in another way and ask, if a white beam be substituted for the spectral color, how intense will this white beam be, relative to the constant white, when it induces 25 per cent as many positive responses as the latter? In other words, what is the relation between the relative intensity of two beams of white light and the relative magnitude of the responses induced by them?

This relation was found without much difficulty. The apparatus used for this purpose was the same as that represented in Figure 1, except that for the spectroscope unit there was substituted another unit just like the comparison lamp and its container, so as to produce two beams of white light against the diffusion screens. The beam coming from the comparison lamp was left constant, while that coming from the new unit was varied in intensity through eight steps, ranging from approximately three times the intensity of the comparison lamp down to zero, either by substituting a more powerful lamp, by changing the distance of the lamp, or by interposing a neutral tint filter of carefully measured transmission, or, finally, by turning off the lamp entirely.

The experiment was conducted practically as has been described; that is, the bees, after being placed in the box and fed, were caused to assemble at the opening illuminated by the small pilot lamp (fig. 1, *c*), then the two lamps illuminating the pieces of ground glass at the opposite end of the box were turned on and the pilot lamp was turned off, after which the bees entering each of the two tunnels leading to the ground glasses were counted. The bees were now

again brought together at the starting point, by turning on the pilot lamp and extinguishing the other two; then the pilot lamp was turned off and the other lamps on, after which the bees entering each tunnel were again counted. The cycle was repeated again and again, new bees being used from time to time, as already described, until a total of from 500 to 2,000 bees had entered the two tunnels for each of the eight values of the variable illumination used.

Since the purpose of this experiment was to find the relation between the relative intensity of the variable beam and the relative number of bees going toward the spot illuminated by it, the results are tabulated as two ratios: (1) The ratio of the intensity of the variable to that of the constant beam, and (2) the resulting ratio of the number of bees attracted by the variable beam to the number attracted by the constant beam. The intensity ratios, for the eight different intensities of the variable beam used, are 2.98, 1.00, 0.50, 0.25, 0.11, 0.05, 0.01 and 0; the ratios of the effects on the bees corresponding to these are 1.67, 1.01, 0.85, 0.66, 0.50, 0.40, 0.20, and 0.09. These ratios and a summary of the observations on which they are based are presented in Table 2.

TABLE 2.—*The ratios of intensities of a variable beam of light to the intensity of a constant beam, tabulated for comparison with the corresponding ratios of the numbers of bees attracted to each light*

Ratio of intensity of variable beam to that of constant beam	Total number of bees observed	Number of bees attracted by variable beam	Number of bees attracted by constant beam	Ratio of number of bees attracted by variable beam to number attracted by constant beam
2.98	1,000	625	375	1.67
1.00	1,000	502	498	1.01
.50	1,000	459	541	.85
.25	1,500	597	903	.66
.11	1,000	333	667	.50
.05	2,000	576	1,424	.40
.01	500	85	415	.20
0	500	40	460	.09

The data presented in Table 2 show clearly the following facts: When the two intensities of the pair are equal (column 1, ratio 1.00) the stimulative effect produced by each is practically the same (last column, ratio 1.01), as one would expect. But when the intensity of the variable beam is changed, the effect does not change proportionally; for example, when the intensity of the variable beam is decreased so that its ratio to that of the constant beam is 0.11 (first column), the effect produced decreases only to a ratio of 0.50 (last column). For unequal illumination the departure from direct proportionality is evident throughout. It will be observed that even when the intensity of the variable beam is reduced to zero (lamp turned off entirely), some bees enter this unlighted tunnel. This behavior is due partly to the activities of indifferent bees—bees which, after being confined for a little while, seem to lose their photopositiveness and simply roam aimlessly about, sometimes entering the unlighted tunnel; it is due partly also to the flaring shape of the

tunnels, which sometimes catch bees coming in from one side of the box toward the tunnel on the opposite side.

The relation between intensity of illumination with white light and magnitude of response on the part of the bees is brought out more clearly when the relative magnitude of response is plotted as a function of the relative intensity of the variable illumination which produced it. The resulting curve (fig. 2) is not a straight line, but is suggestive of a parabola; however, if the logarithms of the related variables are plotted, an approximately straight line results which correspondingly represents the equation

$$\log R = m \log I + b,$$

where I is the ratio of the intensity of the variable white light to that of the constant white light, R the ratio of the number of bees attracted by the variable light to the corresponding number attracted

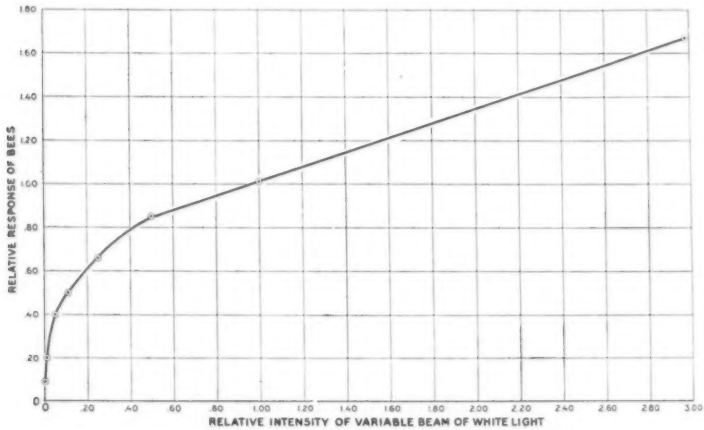

FIGURE 2.—Correlation of the relative intensity of a variable beam of white light and the relative number of bees apparently responding to it

by the constant light, m the tangent to the angle of inclination, and b the y intercept (a constant).

It may be pointed out, in passing, that neither does a straight line result when the relative response is plotted as a function of the logarithm of the relative intensity instead of the relative intensity itself; the resulting curve, shown in the lower part of Figure 3, makes it evident that the relation considered here is not in agreement with the Weber-Fechner law, according to which the magnitude of response increases as the logarithm of the stimulus. Only when the logarithms of both the relative intensity and the relative response are plotted does an approximately straight line result. This approximate proportionality between the logarithm of the stimulus of white light and that of the corresponding response on the part of the bees, a kind of by-product of the present research, seems worth a passing allusion for its mathematical interest. The line would have been perfectly, instead of approximately, straight if the curve in Figure 2 had been

exactly a parabola, and would have been inclined at a different angle. For the greater convenience and simplicity of plotting logarithms which are strictly positive quantities, and inasmuch as only the *relative* values of the intensities of light and of the observed reactions are concerned, the ratios plotted in Figure 2 have throughout been multiplied by 100, and the logarithms of the resulting products are those plotted in Figure 3.

It now becomes possible by the use of the curve shown in Figure 2 to find by graphic interpolation how intense a beam of white light must be, relative to the intensity of the beam from the comparison lamp (fig. 1), in order to produce the same effect as that produced by any one of the spectral colors. The ratio of response induced by the

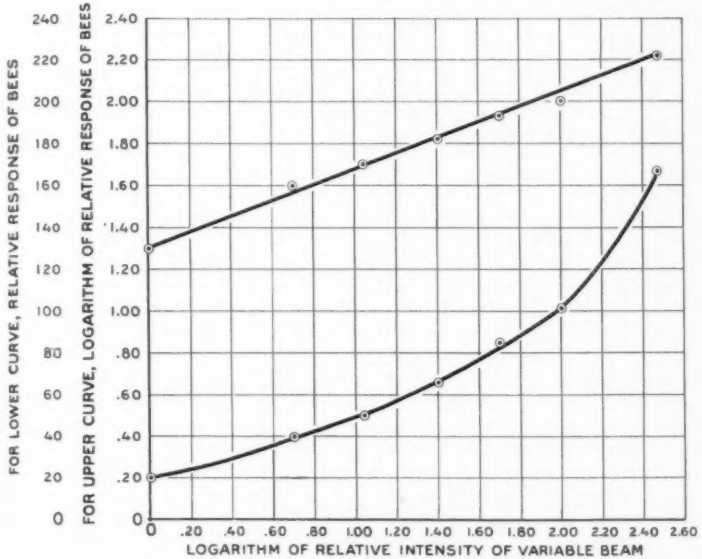

FIGURE 3.—Lower curve, relative response of bees as a function of logarithm of relative intensity of variable beam of white light; upper curve, approximately straight line found by plotting logarithm of relative response of bees as a function of logarithm of relative intensity

spectral region, for example, of wave length 451 mμ is found from Table 1 to be 0.25. Locating this value on the y axis, the corresponding relative intensity of white light is found by the curve to be approximately 0.022, as read off on the x axis. Similar values for the light of each of the 14 spectral bands used in this research were found by this method from a carefully drawn curve like the one in Figure 2 but made on a suitably large scale. In Table 3 are shown, in the first column, the wave lengths of the 14 spectral bands; in the second, the relative stimulative effect of their several colors, taken from the last line of Table 1; and in the third, their relative stimulative values in terms of the constant white illumination, as found by the method of graphic interpolation just described.

TABLE 3.—*Steps in the derivation of the relative stimulative efficiency of selected spectral colors in producing reactions of bees*

Wave length	Relative stimulative effect [a]	Relative stimulative value [b]	Relative energy at slit of spectroscope [c]	Relative stimulative efficiency	Relative stimulative efficiency, with maximum at 100
Mμ				*Per cent*	*Per cent*
431	0.16	0.005	0.18	2.78	10.11
451	.25	.022	.4	5.50	20.00
471	.40	.060	.8	7.50	27.27
492	.57	.163	1.2	13.58	49.38
512	.73	.330	1.8	18.33	66.65
532	.84	.525	2.5	21.00	76.36
553	1.00	.990	3.6	27.50	100.00
574	.91	.700	4.7	14.89	54.15
595	.74	.345	6.4	5.39	19.60
615	.52	.125	8.0	1.56	5.67
636	.29	.030	10.1	.30	1.09
657	.19	.009	12.8	.07	.25
677	.15	.004	16.0	.025	.09
698	.085	0	19.5	0	0

[a] From last line of Table 1.
[b] Found by interpolation on the curve of relative response as a function of relative intensity of illumination. (Fig. 2.)
[c] Determined by W. W. Coblentz.

The values last named are those which are necessary for ascertaining the relative *efficiency* of each of these spectral colors, these relative efficiencies being the ultimate object of the research. They are found by dividing the relative stimulative values shown in column 3 by the relative expenditure of energy in the corresponding spectral color; the several quotients represent the relative efficiencies sought. The relative expenditure of energy in each of the 14 wave lengths used was determined for the writer by W. W. Coblentz, of the United States Bureau of Standards, with a delicate thermopile and galvanometer, and the results are shown in the fourth column of the table. In the fifth column are shown the relative stimulative efficiencies sought, the several quotients having been multiplied by 100 in order to be shown as percentages, and in the sixth column the same relative efficiencies after the maximum efficiency has been given an arbitrary value of 100 and the others a proportionate value; in both these columns the values are given as percentages. It will be seen that the efficiency is very low in the red, not attaining a value of 1 per cent of the maximum until the wave length 636 mμ is reached; from there it rises rather rapidly to a maximum at 553 mμ; from this point it decreases more gradually until at 431 mμ, the shortest wave length obtainable with the spectroscope used, the efficiency is approximately 10 per cent of the maximum.

The distribution of relative stimulative efficiency in the spectrum is made much more evident if these values are plotted as functions of their respective wave lengths and a curve drawn connecting the points. (Figure 4, solid line.)

A comparison of the curve for the bee with that for man, presented in Figure 4, shows that their respective maxima are at about the same wave length, but that the efficiency of the longer waves is greater for man than for bees, and the efficiency of the shorter waves less. The curve for man, as shown in the illustration, has been drawn from

data compiled by Gibson and Tyndall (*12, p. 173*), of the United States Bureau of Standards.

CONCLUSIONS

The upper limit of the spectrum for the honeybee extends to at least wave length 677 mμ. Kühn (*22, p. 799*) found evidence of its extent to only about 650 mμ. The difference between these assigned limits is probably due to the difference in intensity of the spectral light used in the two sets of experiments. The lower limit was not ascertained in the research here reported, but according to Kühn it extends to at least 313 mμ.

The point of maximal stimulative efficiency is at about 553 mμ, which corresponds rather closely with that for man. From this point the efficiency decreases more rapidly for bees than for man toward the

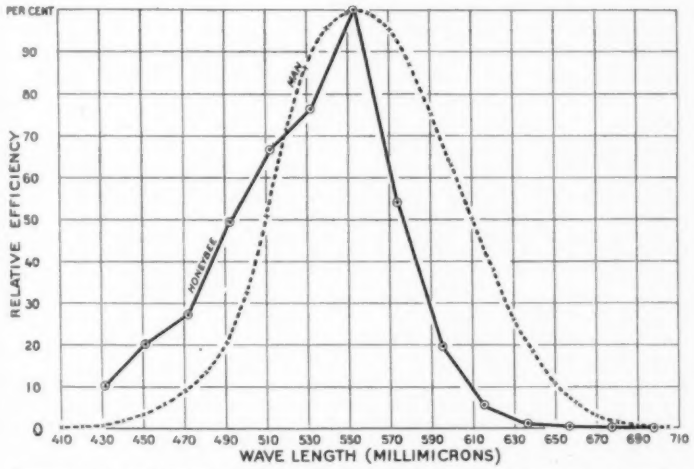

FIGURE 4.—The distribution of relative stimulative efficiency in the spectrum for the honeybee compared with similar distribution in that for man

longer wave lengths, but more slowly than for man toward the shorter wave lengths, being at 431 mμ still fully 10 per cent of the maximum.

Untrained bees, when allowed to walk toward two sources of light of the same quality, placed near together at the end of a rectangular box, go to the brighter more often than to the fainter, but not in direct proportion to the relative brightness of the two. The curve representing the relation between relative brightness (energy) and relative magnitude of response is a polynomial one, of such form that the curve showing the relation between the logarithms of the two related variables is an approximately straight line.

II. CHROMA VISION OF THE HONEYBEE

DISCUSSION OF TERMS

In almost any paper involving the subject of light and color it is necessary to state clearly the sense in which various terms are to be used. This need arises from the lack of agreement among present-

day workers in the field of optics, human psychology, and general physiology as to the meaning of these terms.

A fundamental distinction is to be made between the terms "light" and "color;" the former is the name of a form of energy, entirely objective, while the latter is the name of a sensation, entirely subjective.

Light has three attributes—brightness, wave length, and purity. Brightness refers to the intensity of illumination as measured in some physical unit such as meter candles or ergs per square centimeter per second. In this paper the term is used synonymously with intensity.

Wave length refers to the dominant wave length of the portion of the spectrum under consideration; in practice it is usually impossible to obtain light of a single wave length. The unit of measurement here used is the millimicron.

Purity refers to the proportion of white light which is, in a given instance, mixed with the spectral light in question; the less the admixture of white light the greater the purity.

Likewise, color has three attributes—brilliance, hue, and saturation. We may, however, combine the last two, as will be done in this paper, and say that color has the two attributes, brilliance and chroma.

Brilliance is that attribute of a color which enables it to be classified as equivalent to some member of a series of grays, ranging between black and white. It is the quantitative aspect of color.

Chroma involves the hue—that is, the redness, yellowness, greenness, blueness, etc., of a color—together with the distinctness or vividness of the hue. It is the qualitative aspect of color.

Color, then, is merely a subjective phenomenon; a sensation that arises from the activity of the photoreceptors and their attached nervous mechanism. It includes the sensations not only of chroma (red, yellow, green, blue, and all combinations of these, for the human eye) but also of gray (the whole series from black to white, including the latter). The meaning of these terms becomes more evident if they are viewed in tabular form. The following arrangement of terms here discussed is a modification of one presented by the Progress committee on spectrophotometry for 1922–3 of the Optical Society of America (*33*):

Light (objective) evokes the sensation of color (subjective).
Brightness evokes the sensation of brilliance.
Wave length, Purity } evoke the sensation of chroma { hue, saturation.

If these definitions are adhered to, it will be seen that the terms "color blindness" and "color vision" lose entirely their usual meanings, the former coming to mean entire blindness, and the latter simply light vision; hence in this paper the terms "chroma blindness" and "chroma vision" will be used to express what is ordinarily meant by color blindness and color vision, respectively.

Chroma vision[7] may be defined, then, as the ability of an animal to distinguish between chromas of the same brilliance.

It may be argued that the terms "color," "brilliance," and "chroma," which describe purely human sensations, should not be used when referring to other animals than man, because we do not know what

[7] The use of the term "chroma" to avoid the ambiguities in the meaning of color, has been advocated by Ladd-Franklin (*25*) and Troland (*35*).

kind of photic sensations they have, if, indeed, they have any. That is true, of course, but as a matter of fact, even among human beings, no individual knows exactly how light appears to another individual, and yet we find the terms convenient in talking about these sensations. Likewise, in this paper, the terms are used only for convenience, and it is to be understood that there is no intention of attributing to insects the light sensations of human beings. When, for example, the writer speaks of bees being able to distinguish a blue from all shades of gray, he means colors that are called blue and gray by human beings, and no assumption is made as to what sensations they give to bees.

REVIEW OF LITERATURE

The method which has always been used in investigating chroma vision of bees is the so-called training method, although several other methods have been used for other animals.[8] The method consists essentially of two procedures: (1) The training of the bee to come for food to a place where food is associated with a given chroma, and (2) the testing of the bee, by some change in the layout of the experiment, to see if it can select from other colors (i. e., chromas or grays) of equal brilliance the particular chroma used in training.

The training of bees to come to almost any desired experimentally arranged set-up is usually not difficult, neither is the evidence that bees select a given chroma from other colors difficult to obtain. But it is very difficult to make sure that at least one of the test colors is of the same brilliance to the bee as the training chroma.

Since chroma vision is, by definition, the ability to distinguish between chromas of equal brilliance, the question persistently arises in a critical review of previous experiments, were the colors used in the tests of the same brilliance to the bees as were the chromas used in the training?

Lubbock (*1*) and his contemporaries clearly established the fact that bees and certain other insects can under certain conditions distinguish one chroma from another, but they did not ascertain whether this distinction takes place on the basis of chroma alone or on the basis of relative brilliance; that is, they did not clearly distinguish chroma vision from brightness vision. Forel, however, recognized the fact that the mere ability to distinguish one chromed object from another is not a proof of chroma vision unless the objects are at the same time equally brilliant to the insect. He attempted (*5, p. 181*) to test the ability of bees to distinguish a given color to which they were trained from a number of grays of different brightness, but so many bees came to his dishes for food that the experiment failed.

Von Frisch (*6, 7*), however, carried out experiments in which this sort of procedure was successful. He set out in the vicinity of a hive a series of colored squares of paper, arranged after the manner of a checkerboard, consisting usually of one chromed paper (of the so-called Hering series), about 15 by 15 cm., and from 15 to 31 gray papers each of the same size but different in brightness, varying in approximately equal steps (for the human eye) from white to black (*6*). During the training period he placed on the chromed paper a watch glass containing sugar water and on each of the grays an empty watch glass. The position of the chromed paper was changed fre-

[8] A list of these methods, with bibliography, is given by Kühn (*21*).

quently to avoid training in reference to location. After a training period of from a few hours to several days the assemblage of squares was exchanged for another with fresh papers and clean watch glasses, all of which, however, were now empty. The result was that the bees came flocking to the chromed paper and practically disregarded the grays. This was also true when none of the papers carried watch glasses, and even when the grays carried watch glasses containing food while the chromed paper carried an empty watch glass. The chromed paper must have stimulated the bees differently, then, from any of the grays which were used, and since the grays varied in brightness from black to white, Von Frisch concluded that this difference in stimulation must have been due to difference in chroma, especially since he found that the bees could not distinguish between the grays.

Severe criticism of Von Frisch's work was made by Von Hess (*17*), who pointed out principally the lack of consideration which Von Frisch has given to the bees' sense of smell. Von Hess went so far as to obtain samples of the different dyes used in the Hering papers and noted that some of them were distinctly different from others in odor—even to man. He made a number of experiments with these papers, in which he used a plate of glass to cover them and claims to have obtained no evidence at all of chroma vision. For example, he made a "spectrum" consisting of 185 chromed papers ranging from red to blue, in small steps, so as to give the appearance of a prismatic spectrum. After "training" (Von Hess always put "Dressur" in quotation marks) bees to yellow for several days, he set out this whole "spectrum" covered with glass, on which he made a streak of honey running down the middle of the "spectrum" for its entire length. Bees did not collect first on the yellow but came indiscriminately and collected more or less uniformly along the whole streak. Von Hess therefore flatly contradicts the conclusion that bees possess chroma vision.

Von Frisch then modified some of his experiments in an attempt to meet Von Hess's criticisms. For example, he eliminated the possibility of training to a particular odor carried by the papers, by covering the whole series with a glass plate; this precaution made no significant change in the results. He even sealed papers within glass tubes and obtained the same results. He again (*7*) concludes, therefore, that bees possess chroma vision.

While this conclusion of Von Frisch thus seems to be founded on sound experimental results, it is valid only if the brilliance of each of the chromed papers was, for the bees, equal to that of some one of the grays. But it is by no means certain that this condition obtains, for it is possible that the chromed papers may have been more brilliant than any of the grays, and, indeed, than even the white, This has been ably pointed out by Lutz (*28*), who recalls a fact stated many times by Von Hess and others, that other animals may not have the same limits of perception in regard to wave length as human beings have. That is, it is entirely possible that the chromed papers used by Von Frisch reflected ultra-violet in so much greater amounts than any of the grays that to an animal relatively highly sensitive to ultra-violet these papers would appear brighter than even the white. As a matter of fact, Lutz shows that bees and several other insects are relatively much more sensitive to ultra-violet than man—a fact that has been suspected ever since Lubbock (*1*) demonstrated the sensitivity of ants

to ultra-violet. Lutz then tested the Hering papers used by Von Frisch and found most of them to reflect some ultra-violet. He did not have samples of the grays to test, hence could make no comparison of the ultra-violet reflection of grays and chromas. The fact that in some experiments Von Frisch used a plate of glass to cover his papers does not entirely exclude the effect of ultra-violet, since ordinary glass often transmits down to the wave length 320 mμ.

Lutz, then, while concluding that Von Frisch is probably correct in ascribing chroma vision to bees, does maintain that he has not proved it. He said:

> Hess may be right in believing that insects are totally color-blind. Probably Von Frisch is more nearly correct in saying that they can distinguish all of the colors except red and certain greens as colors * * *.

Kühn (*20*, *22*) performed some experiments designed to meet such criticisms as those made by Lutz. He made several tests, one of the simplest of which may be described as follows: Bees were trained to come for food into a semidark room and there to congregate at a narrow trough filled with sugar water and illuminated from above by a narrow streak of spectral light—e. g., from the blue-green region. After a sufficient period of training, the bees congregated on this streak of light even when the trough of food was removed. If now another streak of light of the same wave length but different intensity was thrown diagonally across this streak, after the manner of the letter X, the bees always collected on the more intense of the two streaks, no matter to which they had been trained. But when a streak of white light, taken directly from the same source as that used to produce the spectral streak, was thrown across the training streak, the bees did not collect on the white but continued to collect on the spectral streak. This he regards as proof that the spectral streak was for the bees qualitatively different from the white streak. But this is true only if the white streak was more brilliant to the bees than the other. On first thought it appears obvious that a beam of white light should always be more brilliant than any of the monochromatic constituents of that white beam. But Lubbock (*1*) long ago demonstrated that this does not necessarily obtain; he showed that white light after it has been modified by being passed through a certain filter is more stimulative to Daphnia than the same white light unfiltered. Hence it is possible that in Kühn's experiment the bees continued to flock to the spectral color merely because it was more brilliant to them than the white; in other words, it had a greater quantitative effect. From these facts it seems evident that the problem as to the presence of chroma vision in bees is open to further investigation.

METHODS AND RESULTS

In order to test the presence of chroma vision in bees it was decided to do three things: (1) Select a series of filters transmitting different portions of the spectrum, (2) ascertain the relative brilliance to bees of the light transmitted by each, and (3) ascertain by experiments in training whether or not bees can select a given one of these chromas from the others after all have been made equal in brilliance.

The filters selected were four made of glass and a fifth, consisting of a glass cell 3 cm. deep, filled with a solution of potassium dichromate (24 gm. per liter). The transmission curves of these filters are presented in Figure 5. The height of the peak of each curve repre-

sents its relative total transmission,[9] and the points of intersection of each with the base line represent with fair accuracy its limits of wave length as determined by observing through a spectroscope the lines of the mercury-vapor spectrum which it transmits,[9] and by the use of spectrograph photographs (*10*). The shapes of the various curves are only approximate, since the transmission for various wave lengths was not determined, but the shapes agree with those of curves for similar glasses, or even identical glasses but of different thickness, as given, for example, by Gibson, Tyndall, and McNicholas (*13*). Since all of these filters when used alone transmit a large proportion of infra-red rays, a cell containing a solution of copper sulphate (19 gm. per liter) 3 cm. deep was always used in connection with them to screen out these rays. A curve for the combined solutions of copper sulphate and potassium dichromate is given by Gibson (*11, p. 274*).[10]

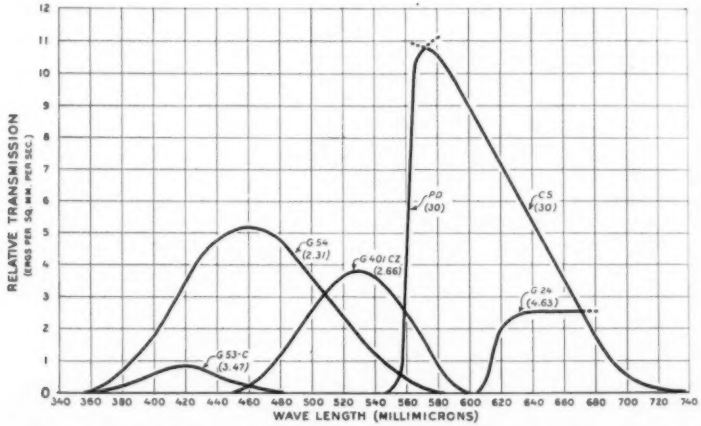

FIGURE 5.—Transmission curves for the color filters used in the study of chroma vision in bees: G53–C transmits violet; G54, blue; G401CZ, green; and G24, red; PD is the curve for potassium dichromate and CS that for copper sulphate. The dimensions in parentheses are the thicknesses (in millimeters) of the filters used

It is, of course, very difficult to find five color filters all of which transmit entirely separate and distinct portions of the spectrum. It will be observed here, for instance, that the yellow filter transmits at the same time all that the red filter does, and the blue all that the violet does. And yet these filters appear unmistakably different to the normal human eye. The reason the blue appears different from the violet is, obviously, because the major portion of the light transmitted by one is in the region we call blue and by the other in the region we call violet. If a bee can distinguish one of these from the other after they have been made equal in brilliance to the bee, we may say with confidence that the bee possesses chroma vision, for even though the transmission curves overlap, whatever difference there is, under these conditions, must be due to chroma.

[9] Kindly determined for the writer by R. Stair in the laboratory of W. W. Coblentz.

[10] The curve in the reference cited is for a solution of copper sulphate ($CuSO_4.5H_2O$) containing 57.0 gm. per liter and for a solution of potassium dichromate ($K_2Cr_2O_7$) containing 72.0 gm. per liter, each in a cell 10 mm. thick. Since in the present investigation the glass cells were 3 cm. thick, the concentration was reduced by two-thirds, giving, according to information personally furnished the writer by Gibson, the same curve as that shown in the reference.

The chromas having been selected, the next problem was to find their relative stimulative effect (relative brilliance) for the bees. One of the main difficulties in doing this proved to be the finding of a suitable reaction to measure—by no means an easy task. It was found, for instance, that when the bees were placed in the intersection of two wide beams of light their reactions could not be measured accurately because of the bees' excitability and rapid movements. Likewise, the ratio of the numbers that congregated at the two ends of a box into each end of which a beam of light entered—although this method was used by Von Hess (*18*) with apparent success—was found in these experiments not to be a sufficiently delicate measure of the relative brilliance of the respective beams, because of the great restlessness of the bees and the ready modification of their behavior through inability to escape, and other incidental factors.

A much more satisfactory measure of the relative stimulative effect was found in the reactions of individual bees to two beams of light coming from opposite directions, as these reactions were observed in the apparatus now to be described. (Fig. 6.) A beam of light from a 400-watt projection lamp passes first through a solution of copper sulphate (to remove the infra-red rays) and then through a pair of rotating sectors, one variable in position with respect to the other. (Fig. 7.) The upper half of the beam is intercepted by only the larger of the two rotating disks *d* but the lower half is intercepted by both. When the sectors of the two disks are made to coincide with each other and are then rotated together, the light intensity of the lower half and that of the upper half of the beam are identical; but by turning the smaller disk *c* with respect to the larger and rotating them together the intensity of the lower half of the beam can be diminished to any desired degree until, when the sectors are exactly opposite in position, it is completely screened out. The position of the smaller disk with respect to the larger can be varied by means of a suitable knurled collar, graduated in percentage.[11] After passing the sectors (fig. 6), the upper half of the beam is deflected by a mirror and proceeds at an angle of 90° to its former direction, while the lower half of the beam is allowed to pass on. The two beams, now proceeding in directions at right angles to each other, are caught at equal distances from the first mirror by other mirrors which turn

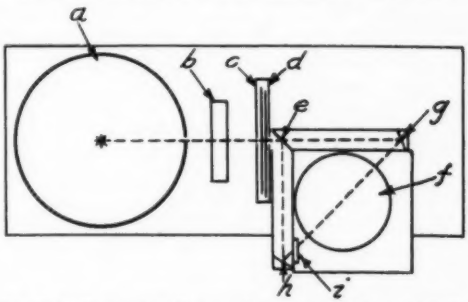

FIGURE 6.—Explanatory diagram of apparatus used to ascertain the relative stimulative effect on honeybees of transmitted light of various chromas: *a*, 400-watt lamp, the source of illumination; *b*, glass cell containing a solution of copper sulphate, for screening out infra-red rays; *c*, *d*, two rotating sectors, adjustable with respect to each other, for transmitting two beams of light of variable relative intensity; *e*, mirror for reflecting one of the two beams at a right angle; *f*, cover (inverted) of a large Petri dish, for containing bees under observation; *g*, *h*, mirrors for reflecting the two beams into two opposite sides of the inclosure *f*; *i*, light filter

[11] The rotating sectors and the lamp house of this apparatus are a part of the Keuffel and Esser color analyzer.

them directly toward each other. The beams now pass through light filters, if desired, and enter the opposite sides of an inverted cover of a large Petri dish, under which are imprisoned from one to three bees. Since the apparatus is set up in a dark room, with the lamp house well shielded, these two beams are practically the only light which strikes the bees. Figure 8, from a photograph, shows the apparatus as a whole, except the Petri dish and the top of the lamp house.

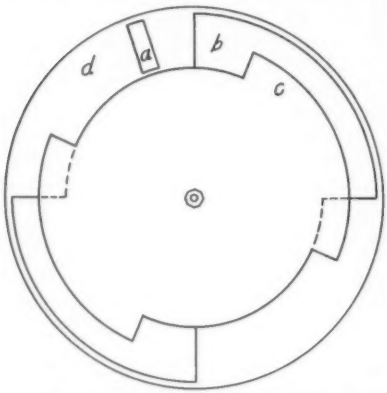

FIGURE 7.—Diagram of rotating sectors used to vary the relative intensity of the upper and lower halves of a beam of light: *a*, The aperture through which the beam passes; *b*, the larger rotating disk, which always intercepts the whole beam, reducing its intensity by one-half; *c*, the smaller rotating disk, its position variable with respect to the other, which intercepts only the lower half of the beam of light, reducing its intensity to any additional degree desired, according to its position with respect to the larger disk; *d*, the housing which incloses the disks

It was found that under these conditions the bees walk hurriedly around under the glass dish, and whenever they come to the spot on the side where a beam of light enters they pause and start as if to walk up the side of the dish. After an attempt or two they pass on until they come again to a spot where a beam enters. When the two beams are decidedly unequal in intensity the bees attempt to escape most often at the more intense beam; by increasing the intensity of the weaker beam, however, a point is reached at which this beam induces about as many of

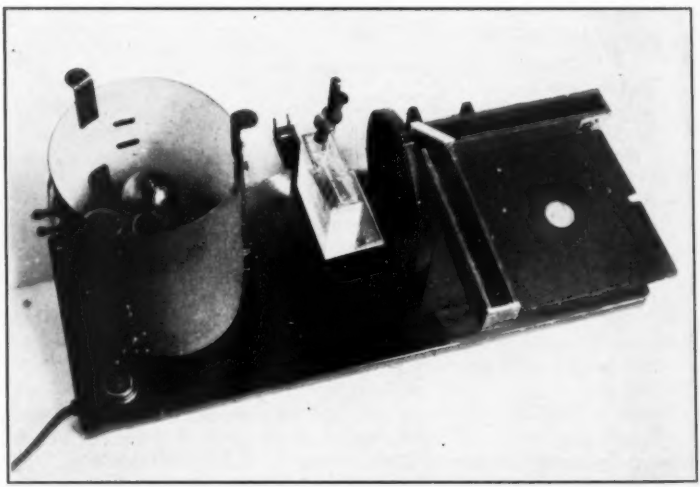

FIGURE 8.—General view, from a photograph, of the apparatus illustrated in Figure 6. The Petri dish and the top of the lamp house are lacking

these peculiar reactions as the other. This is then considered the point at which the two are equal in brillance for the bee.

The procedure, then, was first to place one of the chromed[12] filters, mentioned above in the text and in connection with Figure 5, in the constant beam, leaving the variable beam white. A count of the reactions to each then indicated whether the chromed or the white beam was the more stimulative to the bees. If the white was the more stimulative its intensity was reduced, if the less stimulative, it was increased, after which, in either case, a count was again made. In this way, after making a series of tests, a percentage of the total white available could be found which induced approximately the same number of reactions as a given chromed beam. This percentage, obtained for each filter, was regarded as a representation of the relative stimulative effect (relative brilliance) of the light produced by each filter.

It was found that the exact percentage of white required to equalize a given chromed beam in stimulative effect was very difficult to ascertain accurately. The difficulty may have been due to an insufficiently delicate means of measuring the reactions, but it was probably due rather to an inability of the bees themselves to recognize differences in brilliance between a chromed and a white beam unless those differences are of considerable magnitude.

In the method finally chosen for finding these values the percentage of available white used was first made so low that in most tests it definitely induced fewer reactions than the chromed beam. Then the intensity of the white was increased by small steps until it definitely induced more reactions than the chromed beam. By putting down in order the results of these tests, a point could be chosen which seemed to represent with fair accuracy the percentage of white which just equaled the chroma in stimulative effect. Thus, in the experiment in which green was compared with white, white at the intensity of 15 per cent of the total white available induced 48 per cent of the total reactions; at the intensity of 20 per cent it induced 45 per cent of the total reactions; and with the percentage of white increased at suitably chosen steps, fully set forth in Table 4, the percentage induced of the total reactions increased correspondingly to 58 per cent, both for 40 and for 56 per cent of the maximum available white. In this series it appears that any percentage of white between 15 per cent, or perhaps lower, and 35 per cent was approximately as stimulative to the bees as green. There is an indication of a gradual rise, however, so that the most probable value for equal stimulative effect seems to fall at from 28 to 30 per cent, and the latter figure was adopted.

These results and those obtained for the other chromas are presented in Table 4. The percentage of white required to equal the effect of blue seemed to lie between 7 and 25, with the mean at about 20, and for the yellow the percentage seemed to lie between 15 and 30, with the mean also at about 20. For the violet and red the brilliance was very low and hard to measure. For the former the mean percentage of white required was about 2, and for the latter between one-fourth and one-half, and the larger fraction was chosen.

[12] In accordance with the definition of chroma discussed at the beginning of Part II, a chromed filter, chromed beam, etc., is any filter, beam, etc., whose color is some other than a shade of gray.

TABLE 4.—*Summary of experiments performed to find the relative percentage of white light required to equal in stimulative effect the light transmitted by five light filters*

Color of filter	Available white used	Bees used	Reactions	Reactions induced by white light	Color of filter	Available white used	Bees used	Reactions	Reactions induced by white light
	Percent	*Number*	*Number*	*Per cent*		*Percent*	*Number*	*Number*	*Percent*
Green	15	6	500	48	Yellow	15	5	600	51
	20	5	150	45		* 20	6	500	47
	25	2	150	47		25	10	1,200	51
	28	4	300	48		30	4	550	53
	* 30	5	500	51		35	6	650	53
	35	10	750	51		40	4	350	52
	40	2	50	58		50	2	150	59
	56	2	50	58		1	8	350	41
Blue	7	3	200	49	Violet	* 2	10	725	55
	10	3	200	50		5	1	50	66
	15	9	550	50		¼	2	25	28
	* 20	3	500	49	Red	* ⅓	8	275	62
	25	9	825	50		1	2	50	82
	30	4	350	55		7	2	25	84
	35	2	200	52					
	48	3	100	56					

* Chosen as being equal to the chromed light in each series.

Multiplying these percentages by 2 to eliminate the fraction, we may say that the relative brilliance of these chromas for bees stands approximately in the numerical ratio of green 60, blue 40, yellow 40, violet 4, and red 1, with the understanding that these are merely mean values. They do not represent the relative efficiency of the chromas considered, but rather the relative stimulative effect of the chromas on untrained bees.

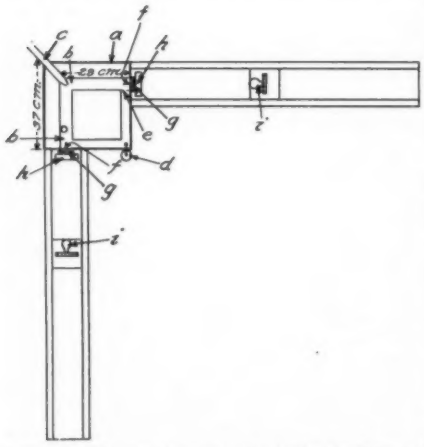

FIGURE 9.—Diagram of apparatus for testing the ability of bees to distinguish between chromas: *a*, Box 37 cm. square; *b*, *b*, glass-covered tunnels, about 3 cm. wide, by 2 cm. deep, within and along the sides of a square 28 by 28 cm. outside; *c*, passage between tunnels and exterior of building, for entrance and exit of bees; *d*, feeder; *e*, wire screen; *f*, *f*, diffusion screens of ground glass, each covering a rectangular aperture 15 by 30 mm. in size; *g*, *g*, light filters; *h*, *h*, glass cells for holding solution of copper sulphate; *i*, *i*, 100-watt electric bulbs, for illuminating apertures covered by the diffusion screens, adjustable for distance by means of carriers sliding on tracks

After these values had been found, the next procedure was to ascertain, by training and testing, whether or not bees can select any given one of these chromas from any other one when the two are made equal in brilliance; that is, equal in equivalent percentages of the available white light used with the apparatus just described.

The apparatus (fig. 9) used for this purpose consisted essentially of a square box, measuring 37 cm. on each side, with a passage for the entrance and exit of bees in one corner and a feeder in the opposite corner. Two glass-covered passageways led from the entrance in opposite

directions, leaving at right angles to each other, and following the sides of the box around to the feeder. At a point in each of the two opposite sides of the box, visible from the entrance, a rectangular opening 15 by 30 mm. was made, through which a beam of light entered from a 100-watt lamp placed in a covered tunnel outside. From the inner end of the entrance the two openings were visible in directions at right angles to each other. The intensity of either beam could be varied by changing the distance of the corresponding lamp. A ground-glass diffusion screen was placed over each rectangular opening; as the openings were immovable and constantly equal in size and in distance from the inner end of the entrance, any movement of the lamps caused no variation in the size of the images. The chromed filters were placed just back of the diffusion screens. The whole apparatus was placed in a dark room, and only a narrow tunnel connected it with the exterior. Enough light came from the lamps through the light filters and diffusion screens to enable an observer to watch the bees in the tunnels near the entrance.

The first step in using the apparatus was to get the bees voluntarily to enter the box. This required considerable patience but offered no great difficulties. A dish of sugar water was placed out, preferably in the early morning, until it was found by bees. The first visitor, on her return to the hive, stimulated a number of her companions to rush out in all directions, and some of them came to the food. On their return to the hive, they stimulated others, and in a short time a large squad was built up, the members of which made regular trips between feeding dish and hive. Now the dish was moved toward the training box, at first only a few centimeters at a time, then, as the bees became accustomed to searching (apparently) for it, several paces at a time, letting it remain in each new location until most of the bees had come to it there. When, finally, the entrance leading to the training box was reached, drops of sugar water were placed consecutively every few centimeters along the circuitous route leading from the exterior to the feeder in the far corner of the training box. As soon as the bees had removed one drop they proceeded to the next, until finally a few succeeded in reaching the feeder. In doing this they had, first, to go from bright daylight into a dark tunnel leading from the outside the box itself, located in the dark room, then turn obliquely and walk down a brightly lighted passageway toward one lamp or the other, then turn at right angles into a dark passageway, and finally reach the feeder in the corner. The return trip involved the reverse of this procedure. This series of reactions is much more complex than the reactions used in taking nectar from a flower under natural conditions, and represents, evidently, a considerable modification in behavior. In other words, the bees following this route were learning a new set of facts, differing considerably from those of their usual experience in the hive and in the open air.

Under these circumstances the few bees that first learned the way to the feeder appeared to stimulate their companions, so that, if the food was abundant, the squad of bees became, after a few hours, so large that it was impossible to count them. And not only that, but they developed the so-called "robbing fever," under the influence of which they seemed to lose their usual well-marked ability to learn; instead, they scrambled over one another in what resembled a frenzy of hit-or-miss efforts to find the feeder. To prevent the coming of

such large numbers, one of the most successful methods found[13] was to reduce the size of the opening of the feeder to such dimensions that only one bee could feed at a time. The feeder devised for this purpose is diagramed in Figure 10. The sugar sirup in the inverted bottle *a* runs down through the tube *b* into the cup *c*. From here the sirup runs around through the smaller tube *d* to the small feeding cup *e* until both cups *c* and *e* are about half full. As soon as the level of the sirup in *e*, and consequently in *c*, rises to such a height that the lower end of tube *b* is closed, the entrance of any more air into tube *b* is prevented, and consequently no more sirup runs out of bottle *a*. As soon, however, as a bee feeding at the cup *e* reduces the level of the sirup in *e* and *c* to a point where more air can enter tube *b*, more sirup runs down from bottle *a* and the level in *e* and *c* rises again. Thus, as long as there is sirup in bottle *a*, there will be a supply also at the cup *e*, and it will be fed down only as fast as it is taken away by the bees.

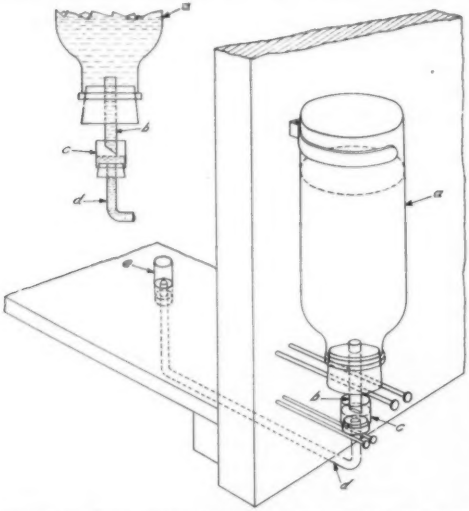

FIGURE 10.—Feeder used in apparatus for testing the ability of bees to distinguish between chromas: *a*, Inverted bottle used as reservoir for sugar sirup; *b*, glass tube conducting sirup into open cup *c*; *d*, glass tube connecting *c* and *e*, the sirup in the two being always at the same level; *e*, feeding cup, made of ¼-inch glass tubing, admitting the head and thorax of one bee at a time

The particular point of interest here is the fact that the cup *e* is large enough to admit the head and thorax of only one bee at a time. This, of course, was an effectual check on the rate at which a squad of bees could obtain food. By the aid of this contrivance and by using a suitable concentration of sugar sirup the rate at which bees came could be reduced almost as much as desired, because the time required for a bee to feed increases with increased concentration. The sirup used in these experiments contained about 2 parts of sugar to 1 of water (by volume). With this arrangement a small squad of bees, usually not more than 15, made regular trips from the hives to the feeder and back, entering and leaving the box at the rate of about one bee a minute. There was an appearance of eagerness and purpose in their behavior, but no frenzy. Usually there were five or so crowding around at all times, ready to enter the opening of the feeder. The bees were at first marked on the back with a mixture of orange G in shellac in order to make it possible to identify the trained bees, but this precaution was found to be superfluous because very few novices came to the box, and these could be distinguished at once by their hesitation and lack of orientation. How-

[13] Suggested to the writer by Jas. I. Hambleton.

ever, the few novices usually learned the route after a while, and their number balanced, approximately, the number that died, so that the squad continued roughly uniform in size.

After a regular squad had been formed and its members were able to find the way to the feeder, a chromed filter was placed behind each diffusion screen and the distance of each lamp from the corresponding screen was so adjusted that the two beams had the same brilliance, according to the values already obtained and recorded in Table 4, and the training then began. Suppose it is desired to train the bees to associate with food the beam of light at the left, as the reader looks at Figure 9. The food is to be obtained only at the feeder in the corner of the box opposite the entrance. In order to reach it the bee, on entering, must walk for half the distance down a passageway directly toward one lamp or the other, then make a right-angled turn and walk an equal distance through a dark passageway until the feeder is reached. But a wire screen is placed in the dark portion of the passageway at the right, so that the bee can finally reach the food only by taking the other passage. After experiencing failure by way of the right but success by way of the left for a period of a day or two, most of the bees take the correct route without hesitation immediately on entering the box. It may be observed that the opening of the feeder is placed in the corner of the box opposite the entrance, so that any odor emanating from the food will be equally present in both passages.

Out of a total number of bees observed as they enter the box, the number taking the correct route—i. e., the route leading to the food—gives the "total per cent trained." They are presumably trained to position, but they may or may not be trained to chroma; that is what is now to be ascertained. To do this the position of the two beams of light is exactly reversed by interchanging the filters and adjusting the distances of the lamps accordingly, but the wire screen is left unchanged. The percentage of the original total number continuing to take the route leading to the feeder after this reversal gives the "per cent trained to position only;" that is, they do not follow the training chroma but continue to go to the old location; they are trained to position rather than chroma. Subtracting the "per cent trained to position only" from the "total per cent trained," we obtain the "per cent trained to chroma."

An example will make the procedure clear: Date, October 21, 1927; training chroma (at left), blue; opposite chroma, violet; length of training period, four hours. At the end of these four hours of training the observer took his stand at the entrance to the box and watched the reactions of 10 bees as they entered. Of these, 8 turned at once toward the blue (i. e., toward the left) and went directly around to the feeder in the far corner. Hence the "total per cent trained" is 80. Now the chromas were interchanged, blue at the right, violet at the left, wire screen still at the right, and the next 10 bees that entered were observed. Of these, 5 turned to the left as the 8 had done before, although now the color at the left was violet instead of blue. Evidently these 5 were not trained to chroma, but to position only; hence the "per cent trained to position only" is 50. Subtracting this latter figure from 80, the "total per cent trained," we obtain 30 as the "per cent trained to chroma."

This method of calculating the net percentage trained eliminates the possibility of including bees which are trained merely to location and not to chroma. It is of course apparent that in this apparatus the bees depend in the training process not only on their sense of sight but also on their sense of direction; that is, they become trained to turn in a *right or left direction* as well as toward a given quality of light. Hence, after a training period, when the position of the two chromed beams is exchanged, no bees will follow the training chroma to its new location unless the association with light is stronger for them than the association with direction. The values obtained, then, for percentage trained to chroma will always represent the training to light which exceeded the training to direction. For this reason, even small percentages of training to chroma are to be regarded as significant.

The results of the 23 experiments performed are presented in Table 5. It will be observed, in the column "Per cent trained to chroma," *that in every experiment except three there was distinct selection of the chroma associated with the food* (the training chroma). That is, after being trained to blue, for example, in the pair blue versus violet, the bees followed this blue when its position was changed, although as far as its nonchromatic brilliance was concerned it must have been indistinguishable to them from the violet. Selection occurred irrespective of which chroma of the pair was used as the training chroma—i. e., as the chroma toward which the bees had to travel during the training period in order to reach the feeder.

TABLE 5.—*Summary of results of a series of experiments performed to test the ability of bees, after they had been trained to associate color with food, to distinguish between chromas of equal stimulative influence*

Training chroma	Opposite chroma	Period trained	Total trained	Trained to position only	Trained to chroma	Average trained to chroma
		Hours	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Blue	Violet	4	[a] 80	[a] 50	30	25
		10¼	80	60	20	
		11½	60	0	60	
Do	Green	2½	80	60	20	
		4	80	40	40	32
		6	70	50	20	
		6	70	50	20	
Green	Blue	10½	100	60	40	40
		7	90	50	40	
Blue	Yellow	8¼	100	87	13	16.5
		9¼	90	70	20	
Red	Blue	8½	100	60	40	45
		8½	80	30	50	
Green	Red	20	80	70	10	20
		11½	80	50	30	
		8¾	70	90	−20	
Red	Green	12½	70	70	0	12.5
		8¾	70	40	30	
		9	90	50	40	
		30	80	80	0	
Yellow	do	40	90	80	10	17.5
		17	80	60	20	
		8	90	50	40	

[a] In nearly all experiments the percentages given in these two columns are based on observation of the reactions of 10 bees. For instance, 80 per cent means that 8 of out 10 bees reacted as indicated.

Of the three experiments tried in which selection did not occur, two were in the series in which the pair red versus green was used and one in the case of yellow versus green. These three experiments

gave a net percentage trained of minus 20, 0, and 0, respectively, minus 20 meaning, of course, that more bees went in the direction that led to food after the chromas were interchanged than went in that direction before. It seems, however, that no particular significance is to be attached to these values (which simply indicate complete lack of training to chroma) because the other experiments in the same series, performed on the next few succeeding days, gave higher values, from 10 to 40 per cent. It is probable, therefore, that these low values are merely accidental variations.

Do these results definitely establish the presence of chroma vision in honeybees? By definition, chroma vision is the ability to distinguish between diverse chromas of the same brilliance. There can be no question as to the ability of bees to distinguish between the chromas used in the experiments just described. (Table 5.) The only objection that can be raised is with reference to the values for stimulative effect of these chromas, found as described above and presented in Table 4. That is, if, perchance, owing to some fault in these tests, the relative brilliance of blue and violet, for example, were not really in the ratio of 40:4 (Table 4) but, say, 30:4, then their equalization on the basis of 40:4 would have left them really different in brilliance and the selection exhibited by the bees could have occurred on the basis of brilliance and not of chroma. But if it were true that the bees selected these chromas on the basis of brilliance alone, then, by varying in both directions the intensity of one member of any given pair of chromas, one ought to find somewhere an intensity at which its brilliance is just equal to that of the other chroma of the pair and produce thus a condition in which no selection would occur.

A series of experiments of this kind was performed, using the chromas yellow and green, which, according to Table 4, have a relative brilliance of 20:30. In the first experiments the chromas were equalized on this basis (lamps set 32.6 cm. from yellow filter, 40 cm. from green filter) and gave in four experiments percentages of bees trained to chroma of 0, 10, 20, and 40, having an average of 17.5. Then the intensity of the yellow was reduced to 75 per cent of its previous value (distance of lamp increased to 37.7 cm.), and two experiments gave percentages of 20 and 60, or an average of 40. Then the intensity of the yellow was still further reduced, to 44 per cent of its original intensity (distance of lamp increased to 49 cm.), and two experiments gave percentages of 40 and 50, or an average of 45. Finally, the intensity of the yellow was increased to 125 per cent of its original intensity (distance of lamp decreased to 29.2 cm.) and three experiments gave percentages of 15, 10, and 23, or an average of 16. Hence, even though one refuses to accept the chosen stimulative values, in terms of white light, of the chromas considered in Table 4 as accurate, and uses instead various other relative intensities, the result is the same, within the purpose of this research; the bees continue to distinguish between the chromas.

It is therefore maintained that the presence of chroma vision in bees is established.

THE NUMBER OF CHROMAS DISTINGUISHABLE BY BEES

It is interesting to observe, in passing, some work that has been done on the number of different chromas bees can distinguish and the bearing of this work on the problem of the experiments recorded

in this paper. Von Frisch's (*6, 7*) experiments in training bees to come for food to various colored papers led him to conclude that they are able to distinguish only two chromas, the short-wave region comprising the blue and violet and the long-wave region comprising the green, yellow, and light red, and that they are chroma blind to blue-green and dark red. Kühn and Pohl (*24*), using a similar method, but with sections of the mercury-vapor spectrum instead of colored papers, concluded that there are four regions of the spectrum distinguishable by bees—the yellow and green region, the blue-green region, the blue and violet region, and the ultra-violet region. Kühn (*22*), supplementing the mercury-vapor spectrum with the continuous spectrum from a carbon arc, defines these regions more precisely as having for limits the wave lengths 650 to 510 mμ for the red, yellow, and green region, 500 to 480 mμ for the blue-green region, 480 to 400 mμ for the blue-violet region, and 400 to 313 mμ for the ultra-violet region.

On the basis of the experiments recorded in the preceding pages, the writer (*4*) in 1928 contended that bees are also able to distinguish blue from violet and green from light red. In Table 5 of the present paper, for example, blue is shown to be distinguished from violet by 25 per cent of the bees, and green from red by 20 per cent in one group of experiments and 12.5 per cent in another. But the width of the band of spectrum transmitted by the filters used, as shown in Figure 5, prevented him from giving the proof for such a contention, and therefore in writing the present paper no point was made of it. That is, the blue might have been distinguished by the bees from the violet because of the amount of blue-green transmitted by the blue screen, and the green may have been distinguished from the red because of the amount of blue-green transmitted by the green screen. If the contention is true, however, there are altogether six chromas which a bee can distinguish.

But subsequent to the appearance of the paper (*4*) cited in the preceding paragraph, and also subsequent to the writing of the first part of the present paper, there has come to the writer's attention a short article by Kühn and Fraenkel (*23*), in which they state that further work along the line pursued by Kühn (*22*) has shown that in the region comprising red, yellow, and green, and again in that comprising blue and violet, there are three nuances of color qualities distinguishable by bees. Hence the total number of chromas which it is claimed bees can distinguish now stands at eight.

One wonders if further research will not disclose still other chromas in the spectrum which are visible to the honeybee. By way of comparison it is interesting to note that for the human eye, according to Nutting (*32, p. 60–61*), there are "from about 130 to 180 distinctly different pure hues between the red and violet, while from violet back to red through the purples and magentas there are about 20 additional steps as determined by their complementaries in the green."

CONCLUSION

In view of the findings of the research described under "Methods and results" in the present article, it is maintained that the presence of chroma vision in bees is established, and that the conclusion of Lubbock, Von Frisch, Kühn, and others is confirmed. The very fact of chroma vision connotes a diversity of chromas distinguishable by

bees, several of which have been reported by investigators, and it is presumable that the number of such distinct chromas will be augmented by further research.

III. ABILITY OF THE HONEYBEE TO DISTINGUISH DIFFERENCES OF BRIGHTNESS

It seems self-evident that any animal that sees at all must have some ability to distinguish differences in brightness. Particularly ought this to be true for the honeybee, which, as is well known, reacts strongly to light. For this reason one is surprised to find in the literature records of experiments in which it is indicated that bees are entirely unable to distinguish any except relatively enormous differences in brightness. Von Frisch (*7, p. 105*), for example, after arranging in the manner of a checkerboard a series of pieces of paper colored various shades of gray, trained bees to come for food to a given one of these papers; then he rearranged the papers in different order and took the food away to see if the bees would seek out the same shade of gray as that on which the food had previously been placed. But they did not; they went to all the grays. Only when he used a very dark gray or a very light gray for training did the bees exhibit any evidence of ability to pick out the corresponding shade. He concluded, therefore, that bees possess a very poorly developed sense of brightness.

Von Hess (*18*), on the contrary, using a method in which training of the bees was not involved, concluded that they have practically as acute a sense of brightness as does man. In one experiment, for example, he confined bees in a cubical glass box on two opposite sides of which were placed tunnels containing lamps, one of which could be moved within its tunnel nearer to or farther from the box. Between the tunnel and the glass box on each side was placed a piece of white paper to serve as a diffusion screen. When the intensity of the light on the two paper screens was very unequal the bees crowded together toward the brighter, when equal they distributed themselves at random throughout the box. Von Hess observed that when the intensities on the two screens were changed from equality to a ratio of 1.00:0.86 they began to appear different in brightness to his own eyes, and that when the ratio was changed slightly to 1.00:0.83 the bees began to distribute themselves unequally in their container. Other experiments gave similar results, indicating that the bee is only slightly less acute than man in distinguishing differences in brightness.

Kühn (*20*), however, came to a conclusion agreeing with that of Von Frisch. He trained bees to come for food to a narrow trough illuminated by a streak of white light of a given intensity. Then, after they had become well trained, he removed the trough and threw on the table with the original streak of light another streak of different intensity which crossed the former diagonally, the two streaks together thus making a figure like the letter X. When the bees now came searching for food they alighted on the more intense of the two streaks, no matter which was the one used in their training. Kühn (*20, p. 118*) concludes, "Auf eine bestimmte Helligkeit sind die Bienen in diesen Versuchen nicht dressiert und auch nicht dressierbar."

The writer has had occasion to use both the method involving training and that involving merely untrained reactions. By use of the former method he (*4*) obtained evidence which led him to conclude that bees are "trainable" to differences in brightness. Using the same apparatus as that employed in testing chroma vision, described in Part II of this paper (fig. 9), but without the color filters, and the same method of procedure as described there, he was able to show that bees can distinguish two illuminated areas when one is as much as, or more than, 4 times as bright as the other, but probably not when one is only 1.3 times as bright as the other.

By use of a method involving only the untrained (instinctive) reactions of bees, the writer also demonstrated their ability to distinguish differences in brightness. The method used is described in the experiments dealt with in Part I of this paper. Bees were placed at one end of a dark box and allowed to walk toward two illuminated areas situated about 10 cm. apart at the opposite end of the box. In some experiments the illuminations of these areas were made equal in intensity and in others unequal in a variety of widely separated proportions. It was found, in brief (Table 2), that when the intensity of one was decreased (by large intervals) the relative number of bees going to that one decreased also, not in direct proportion but according to a curve (fig. 2) discussed there and of no particular interest here. That set of experiments did, however, establish a method of investigating this problem and confirmed Von Hess's conclusion to the extent that bees do instinctively distinguish between the brightness of two illuminated areas provided they are sufficiently different in brightness, but it did not show the *least* difference in brightness that bees can distinguish. (The ratio given in Table 2 which represents unequal intensities having the smallest difference in brightness is 0.50, which means, of course, that one intensity was half as great as the other.)

What is evidently needed, therefore, is a series of experiments to test in a quantitative way the bees' ability to distinguish differences in brightness.

Moreover, since at the present time there is much interest in comparisons of the acuity of various senses in bees with that of the corresponding senses in human beings, it also seems valuable to make another test of the relative acuity of the sense of brightness in these two. It was commonly believed in former times that the bee's senses are remarkably acute, but most of the results of actual tests have shown that they are less acute than those of man. As to taste, for example, Von Frisch (*9*) finds that the bee appears to be less acute than man for sweet and for bitter; and as to smell (*8*) it is said to be no more acute than man. In regard to sight, bees are far less able to distinguish chromas than we are, there being only eight chromas distinguishable by bees, according to Kühn and Fraenkel (*23*), in contrast to the 150 or so distinguishable by man; and in ability to distinguish details of shape and form they are far less acute than man (*2, 16*); according to Hecht (*15*) they are about one one-hundredth as acute. The question thus persists as to how bees compare with human beings in the ability to distinguish differences in brightness. Are they very much inferior to man, as Von Frisch and Kühn maintain, or practically equal to man, as Von Hess maintains? Although there is available much information on this subject in regard to human

beings, as for example that reported by Nutting (*32*), it can not, in fairness to the bees, be used in this comparison because it has been obtained with much more refined apparatus than can be used with bees.

A series of experiments was made, therefore, to obtain with the same apparatus for both bees and human beings as accurate quantitative measurements as possible of their ability to distinguish differences in brightness. These experiments are now to be described.

METHOD

The apparatus (fig. 11) was essentially the same as that used in a previous series of experiments (Part I). It consisted of a shallow, rectangular box, *a*, covered with wire screen, 67 by 26 by 4 cm. inside, and of three boxes, *b*, *c*, and *d*, for holding lamps. The smallest lamp box, *b*, was placed at one end, designated as the left-hand end, of the large box *a*, the two others at the opposite or right-hand end. Light from the lamp in box *b* illuminated a small area of a ground-glass screen *e* placed in the center of the left-hand end of the large box *a*, and the lamps in boxes *c* and *d* illuminated areas 6.5 by 13 mm. on the

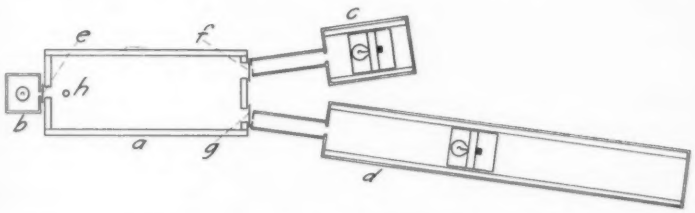

FIGURE 11.—Diagram of apparatus used in studying the ability of bees and of human beings to distinguish between small differences of brightness. See text for explanation

ground-glass screens *f* and *g*, placed equidistant from the corners at the right-hand end of the large box *a*. A hole in the floor of the large box in the mid-line near the left-hand end permitted the entrance of bees. The whole apparatus was placed in a dark room. Box *b* contained a 15-watt lamp, the boxes *c* and *d* either 10-watt or 75-watt lamps, always both the same in any one test. The lamp in box *c* remained always at the constant distance of 40 cm. from the screen *f;* the lamp in box *d* was placed either at 40 cm. from screen *g*, where the intensity on the screen was equal to that produced by the lamp in box *c*, or at 42.1 cm., 44.7 cm., 48.0 cm., 51.6 cm., 56.0 cm., 63.2 cm., or 73.0 cm., where the intensity was reduced, respectively, to 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, or 0.3 the value of that produced by the lamp in box *c*. The lamps in boxes *c* and *d* were interchanged at the middle of every experiment to neutralize any possible difference in brightness between the two lamps. The lengths of the wires leading from the switch to the lamp sockets in *c* and *d* were made equal, in order to obviate any difference in brightness due to differences in resistance.

In performing an experiment with this apparatus, a bee was captured at a feeder,[14] in a small screen-wire cylinder about the size of an ordi-

[14] Field bees must be used. Hive bees that have not yet begun to collect from the field are not positive to light.

nary test tube, taken inside the dark room, and fed with sugar water (by inverting over the screen-wire cylinder a small vial containing this liquid, its mouth covered with a layer of cheesecloth) until it was satiated,[15] then it was released into the large box *a* through the hole *h* in the floor. At the same time the lamps in boxes *c* and *d* were turned on, so that the bee on entering found itself confronted by two illuminated areas 60 cm. away and 10½ cm. apart. Sufficient light diffused from the illuminated areas to enable the observer to see the bee in the box. The bee now walked toward the spots of light, soon veering toward one or the other, and eventually bumping into one of them if allowed to proceed far enough. Ordinarily, however, as soon as it could be ascertained which lighted area the bee was headed for, the lamps in boxes *c* and *d* were turned off and that in box *b* turned on, and the bee immediately turned around and walked toward the source of light in the latter box. When it had come near the hole in the floor through which it had entered, the lamp in *b* was suddenly turned off and the lamps in *c* and *d* turned on, whereupon the bee turned around and walked again toward the right-hand end of the box, going toward one or the other of the areas illuminated by the lamps in *c* and *d*.

This marching and countermarching was continued until, for most of the tests, the bee had made five trips toward the right-hand end of the box. Detailed records were kept as to which area it went on each trip. Such a record, giving the responses of 20 bees to areas whose intensities of illumination had the ratio of 0.3:1.0, is presented in Table 6. In the summarized results records were made (1) of the total responses of all bees to each pair of illuminations and (2) of the sum of the initial responses of individual bees to a pair of illuminations. It was thought that the initial response of the bee when first faced with these two illuminated areas might better indicate which area really appeared the brighter to the bee than the sum of five reactions, but this idea does not appear to be correct.

After one bee had made five successive trips it was taken out into the open and released and another bee captured and given the same treatment. Experience had previously shown that after five such trips many bees become sluggish, and begin to wander aimlessly about the box, apparently having lost much of their positiveness to light.

For most of the tests a total of 30 bees were used for each pair of illuminations, giving therefore 150 responses. For some pairs of illuminations data previously gathered were included, giving as many as 1,150 responses for a single pair. Eight pairs of illuminations were employed, the ratios of intensities of which have been given, with two sizes of lamps, making 16 tests in all.

[15] The feeding tends to quiet the bees and prevent them from racing wildly over the box when released into it.

TABLE 6.—*Detailed record of two tests, giving reactions of bees to a pair of illuminations having intensities in the ratio 0.3:1.0* [a]

[Left-hand lamp 10-watt, at a distance of 40 cm.; right-hand lamp 10-watt, at a distance of 73 cm.]

Date August, 1928	Hour	Bee No.	Reactions		Date August, 1928	Hour	Bee No.	Reactions	
7	10.30	1851	· · · · ·	· ·	7	3.35	1871	· · · ·	· ·
7	10.40	1852	· · · ·		7	3.40	1872	· · · · ·	
7	10.45	1853	· · · · ·		7	3.45	1873	· · · · ·	
7	10.50	1854	· · · ·	· ·	7	3.50	1874	· · ·	· · ·
7	11.00	1855	· · · ·		8	9.30	1875	· · · · ·	
7	11.05	1856	· · ·	·	8	9.35	1876	· · ·	·
7	11.10	1857	· · · ·	·	8	9.40	1877	· · ·	·
7	11.15	1858	· · · ·		8	9.45	1878	· · · ·	
7	11.35	1859	· · · · ·		8	9.50	1879	· · · ·	·
7	11.45	1860	· · · · ·		8	9.55	1880	· · · · ·	
			24	26				38	12

[a] This table is an attempt to reproduce as exactly as is practicable in print a small part of the writer's record of the observations described in the text. Each bee used was assigned a number, to the right of which a series of five dots, reading downward, represents the five consecutive reactions of the bee. A dot to the left of the vertical line records the approach of the bee to the ground glass *f* (fig. 11), and one to the right of that line an approach to the ground glass *g*, and vice versa. The hours indicated in this table range from 9.30 a. m. to 3.50 p. m.

For comparison of the acuity of vision of the bee with that of man it is evident that the same or similar apparatus must be used. The abundant data already available for the perception by man of differences of brightness, such as that published by Nutting (*32*) in 1920, obtained with refined apparatus much of which can not be used for bees, would necessarily give man an unfair advantage in the comparison. In these experiments, therefore, human subjects were tested with the same apparatus as was used for bees.

The subjects were brought into the dark room one at a time and placed in such a position that they faced toward the two screens *f* and *g*. After allowing from one to two minutes for adaptation of the eyes to the darkness (corresponding to the time allowed for the bees to feed), the lamps in boxes *c* and *d* were turned on and the subject was asked which spot of light appeared the more brilliant to him. After he had answered, others (a total of from 1 to 11 in different tests) were brought in and tested similarly. Then the relative intensity of illumination by the two lamps was changed and the same or other subjects tested again. Seventy-five-watt lamps were used in all tests, set to illuminate the ground-glass screens at intensities in the ratios 1.0 : 1.0, 0.9 : 1.0 : 0.8 : 1.0, and 0.7 : 1.0. The positions of boxes *c* and *d* were interchanged frequently, and every known precaution was taken to avoid giving the subjects any clues as to which area really was the brighter. Most of the subjects did not even know how the apparatus was constructed until after the tests were completed.

RESULTS

The results for bees are presented in Table 7, and those for human subjects in Table 8. Figures 12 and 13 present graphically the results for bees, the former with illumination by 10-watt lamps, and the latter with illumination by 75-watt lamps. It will be seen that when the illuminations of the two areas were the same the number of responses to each, for both bees and human beings, was the same with the exception of the 30 initial responses recorded for bees when the 10-watt lamps were used. (Fig. 12.) That this exception is due to the small number of bees employed is indicated by the fact that the observation of 1,150 and 1,000 responses, respectively, in the other two experiments with intensity ratio of 1.0:1.0 gave results almost exactly 1.0:1.0. (Table 7.) When the intensity of illumination for the one area was lowered to 0.9 (Table 7, intensity ratio 0.9:1.0) the response of bees to the dimmer illumination also decreased in most experiments, but when the intensity was further lowered to 0.8, the response of bees to the dimmer in all experiments increased again until more than half were going to the dimmer area. (Table 7 and Figures 12 and 13.) This indicates that bees do not distinguish between illuminated areas under these conditions even when the intensity of one illumination is reduced to 80 per cent of the intensity of the other. When it was reduced to 0.7, however, in most experiments the dimmer area attracted fewer bees than the brighter, and when reduced to 0.6, 0.5, 0.4, and 0.3 it attracted on the whole fewer and fewer bees, although the decrease in response of the bees was not directly proportional to the decrease in intensity of the variable illumination.

TABLE 7.—*Summary of experiments to test the ability of bees to distinguish between the intensity of illumination of two illuminated areas, with results for each ratio of intensities used*

AREAS ILLUMINATED BY 10-WATT LAMPS [a]

Variable illumination in terms of constant illumination	Bees used in observing total responses	Total responses observed	Total responses to variable illumination, in terms of total responses to constant illumination	Bees used in observing initial responses	Initial responses to variable illumination, in terms of initial responses to constant illumination
Ratio	*Number*	*Number*	*Ratio*	*Number*	*Ratio*
1.0	100	1,150	1.003	30	1.31
.9	30	150	.90	30	.67
.8	30	150	1.11	30	1.14
.7	30	200	1.06	30	.67
.6	30	150	.67	30	.88
.5	100	900	.77	40	.29
.4	30	150	.79	30	.76
.3	30	150	.61	30	.36

AREAS ILLUMINATED BY 75-WATT LAMPS [a]

1.0	60	1,000	1.008		
.9	30	150	.92	30	1.14
.8	30	150	1.03	30	1.50
.7	30	150	.76	30	.67
.6	30	150	.60	30	.43
.5	30	150	.61	30	.50
.4	30	150	.52	30	.20
.3	30	150	.50	30	.67

[a] For the 10-watt lamp at the distance of 40 cm., the minimum distance used, the intensity of illumination on the screen was approximately 45 meter candles; for a 75-watt lamp at the same distance, approximately 580 meter candles.

TABLE 8.—*Summary of results of experiments to test the ability of human beings to distinguish between the intensities of illumination of two illuminated areas,* [a] *with results for each ratio of intensities used*

Variable illumination in terms of constant illumination	Subjects used	Correct choices	Incorrect choices	Total choices	Correct choices in terms of total choices
Ratio	*Number*	*Number*	*Number*	*Number*	*Per cent*
1.0	2	2	0	2	100.0
.9	12	15	9	24	62.5
.8	9	11	2	13	84.6
.7	8	8	0	8	100.0

[a] Areas illuminated by 75-watt lamps. At the distance of 40 cm. the intensity of illumination was approximately 580 meter candles.

It will be observed in Figures 12 and 13 that for total responses the results are clearer and more consistent (the curves smoother) with the use of the 75-watt lamp than with the 10-watt lamp. For initial responses neither lamp seemed to have any advantage. In comparing total responses with initial responses, however, the total responses give much more consistent results.

It may be concluded, therefore, that under the conditions of intensity, type of apparatus, and adaptation to darkness used here bees

begin to distinguish between two illuminated areas when the difference between their intensities of illumination is increased to that represented by the ratio 0.7:1.0, but do not distinguish between them if the difference is equal to or less than that represented by the ratio 0.8:1.0.

When these results are compared with results obtained with human subjects the difference is striking. When the areas were made equal in brightness the human subjects used could see no difference between them (Table 8), as was to be expected. When the brightness of one area was reduced to 0.9 of that of the other, however, 15 out of 24 subjects, or 62.5 per cent, selected it as the dimmer. When further reduced to 0.8, in 11 out of 13 tests, or 84.6 per cent, that area was

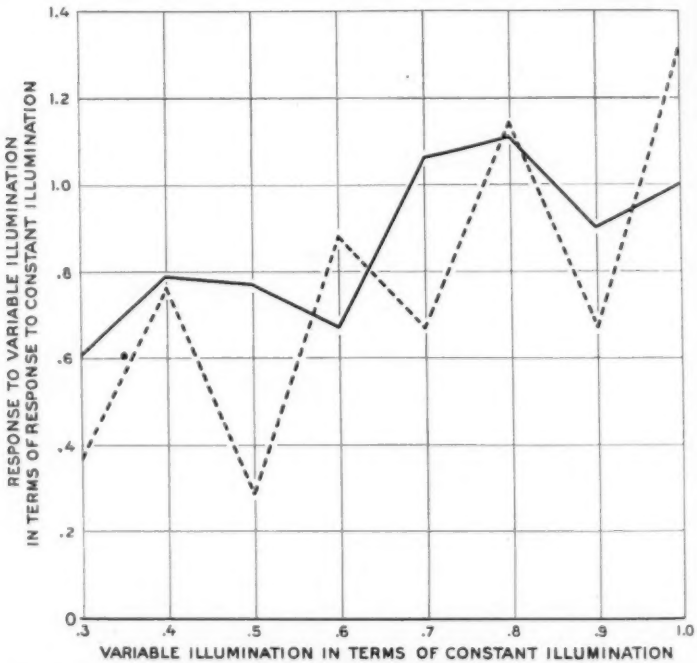

FIGURE 12.—Relative response of bees to illuminations of various relative intensities, when 10-watt lamps were used as sources of illumination. Broken line, averages of initial responses of bees; solid line, averages of total responses

selected as the dimmer, and when reduced to 0.7, all of the eight subjects selected it as the dimmer.

It may be concluded, therefore, that under the conditions of intensity, type of apparatus, and adaptation to darkness prevailing here, human beings can usually distinguish between two illuminated areas when the difference is only that represented by the ratio 0.9:1.0 and can unmistakably distinguish between them when the difference is as much as that represented by the ratio 0.7:1.0; and, accordingly, that human beings are considerably more sensitive than bees to differences in intensity of illumination.

Such a qualitative statement as the above seems amply justified by these experiments, but to state quantitatively how much more sensitive men are than bees requires a great deal of caution, as indeed does the quantitative comparison of the behavior of man with that of any other animal. Caution is necessary here first of all because of a difference in the nature of the response; the human subjects were asked to state which of the two areas appeared brighter (or dimmer) to them, whereas one obtained similar information from the bees by observing their movements. With human subjects the response is simple,

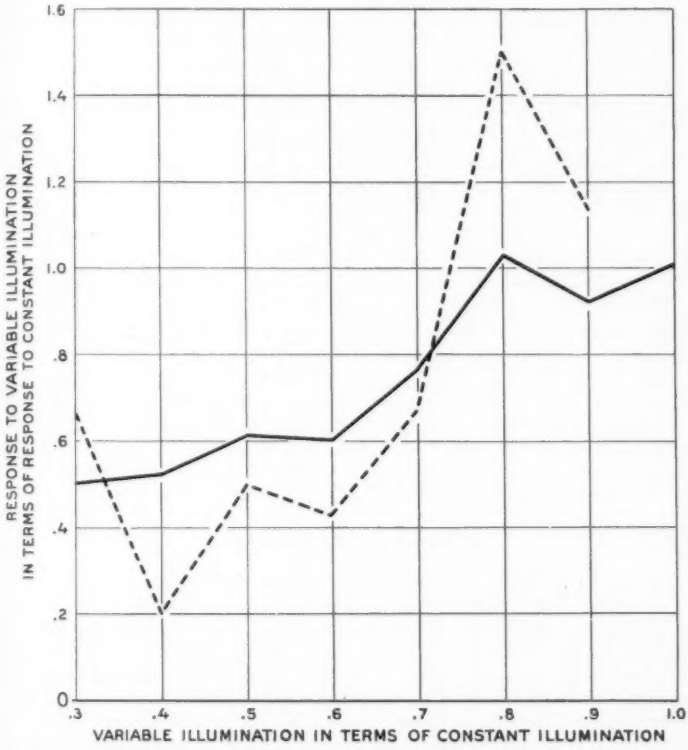

FIGURE 13.—Relative response of bees to illuminations of various relative intensities, when 75-watt lamps were used as sources of illumination. Broken line, averages of initial responses of bees; solid line, averages of total responses

involving only sight and speech in a reaction hardly more than a simple reflex. With bees the response may be enormously complex, since the reaction is in all probability an attempt to escape from the box, which is influenced on occasion by a difference in the intensities of two separate illuminations, and is consequently a very complicated reflex, conditioned not only by the metabolic condition of the bee but by all its past experiences in dealing with such situations.

Caution is necessary, secondly, because of a fact which may be closely correlated with the first; namely, a wide variation in the re-

actions of the bees under identical conditions. One of the most striking examples of this was a test in which reactions were obtained of 10 bees to two intensities whose ratio was 0.3:1.0 (with 10-watt lamps); of the first 50 reactions obtained 24 were toward the intensity of 1.0, while 26 were toward the intensity of 0.3, whereas of the last 50 reactions obtained with 10 other bees, 38 were toward the intensity of 1.0 and 12 toward the intensity of 0.3. If one had stopped with the first 50 reactions it would have appeared that the bees did not distinguish between intensities so widely different as 1.0 and 0.3. The detailed record of these two tests is given in Table 6. Until one can control more factors influencing the reactions of bees in such experiments as these, one must simply use such large numbers that variations will tend to fall equally on each side of the correct value.

Although a definite quantitative comparison of acuity in bees and in human beings in distinguishing differences of brightness is thus not permissible from the results of these experiments, it is clear that human beings are considerably more acute in this respect than bees, and the method here followed offers the possibility of comparing other insects with bees, provided large numbers of each insect are used.

CONCLUSION

From the results of these experiments it appears that neither the conclusion of Von Frisch and Kühn that bees distinguish only enormous differences in brightness, nor that of Von Hess that bees are practically as acute as man in this ability is correct. The more nearly correct conclusion seems to be in the nature of a compromise between the two, namely, that bees begin to distinguish between two illuminated areas when the intensity of one is reduced to at least 70 per cent of the intensity of the other, whereas human beings distinguish between them equally well when the intensity of the one is reduced to only 90 per cent of that of the other.

GENERAL CONCLUSIONS

The upper limit of the spectrum for the honeybee extends to at least wave length 677 mμ in the red. The difference between this result and that of 650 mμ found by Kühn is probably due to a difference in the intensity of the spectral light used in the two sets of experiments. The lower limit was not ascertained in the research here reported, but according to Kühn it extends to at least 313 mμ.

The point of maximum stimulative efficiency for the honeybee is in the yellow-green at about 553mμ, which corresponds rather closely with that for man. From this point the efficiency decreases more rapidly for bees than for man toward the longer wave lengths, but more slowly than for man toward the shorter wave lengths, being at 431 mμ still fully 10 per cent of the maximum.

Untrained bees, when allowed to walk toward two sources of light of the same quality, placed near together at the end of a rectangular box, go to the brighter more often than to the fainter, but not in direct proportion to the relative brightness of the two. The curve representing the relation between relative brightness and relative magnitude of response is a polynomial one, of such form that the curve showing the relation between the logarithms of the two related variables is an approximately straight line.

The presence of chroma vision in bees, already strongly indicated by the work of previous investigators, is more clearly established.

For the moderate intensities used in these experiments (Part III), the bee begins to distinguish between two illuminated areas when the illumination of one is reduced to an intensity 70 per cent as great as that of the other. The ability to distinguish is slightly less pronounced when the intensity of illumination is low (45 meter candles on the brighter area) than when it is high (580 meter candles on the brighter area).

Human beings, tested with the same apparatus as that used for bees (using the higher intensity of illumination), begin to distinguish between the brightness of two illuminated areas when the intensity of one is reduced to 90 per cent of that of the other; this distinction is fairly accurate when the intensity of the one is reduced to 80 per cent, and is quite accurate when the reduction reaches 70 per cent. Human beings are, therefore, considerably more acute than bees in ability to distinguish differences in brightness.

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THE VALUE OF ALFALFA AS A SOURCE OF VITAMIN A IN SORGHUM-GRAIN RATIONS [1]

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INTRODUCTION

In previous papers (*8*, *9*)[2] from this laboratory it was shown that the sorghum grains hegari and yellow milo are strikingly deficient in vitamin A. A quantitative measure of the vitamin A content of these grains and of yellow corn showed that hegari was only one-twentieth as rich in vitamin A as yellow corn. The small amount of vitamin A in hegari was proved to be insufficient for continued growth at a normal rate of young albino rats. All the animals dependent upon hegari for vitamin A exhibited a low degree of health and vigor, with marked susceptibility to infections of various kinds and a striking failure in reproduction and rearing of their young.

As the sorghum grains are drought-resistant and high-yielding, with a minimum amount of water, they are widely used in animal-feeding rations in place of yellow corn, which can not be successfully grown in the arid Southwest. For this reason it seemed advisable to investigate the supplementary value of alfalfa, a crop extensively grown in the Southwest and known to be rich in vitamin A, as a vitamin A supplement to hegari-grain rations.

EXPERIMENTAL METHODS AND MATERIALS

PLAN OF PROCEDURE

Albino rats were taken at the time of weaning from the stock colony reared on Sherman's diet B, composed of two-thirds ground whole wheat, one-third whole-milk powder, and salt equal to 2 per cent of the weight of the wheat, and they were placed upon experimental rations composed largely of hegari supplemented with alfalfa-leaf meal as an additional source of vitamin A and made adequate in other dietary essentials as indicated in preliminary experiments by the addition of casein, yeast, and mineral salts. The composition of these experimental diets is given in Table 1.

TABLE 1.—*Percentage composition of the experimental rations*

Ration No.	Hegari	Corn	Alfalfa-leaf meal	Casein	Yeast	Calcium lactate	Sodium chloride
3	0	73	0	9	[a] 15	2	1
37	73	0	0	9	15	2	1
38	72	0	1	9	15	2	1
39	68	0	5	9	15	2	1
40	63	0	10	9	15	2	1
41	58	0	15	9	15	2	1
42	48	0	25	9	15	2	1
4	71	0	0	9	15	2	[b] 1

[a] The 2 per cent yeast used in the early work was found to be inadequate for continued successful reproduction and lactation.
[b] 2 per cent cod-liver oil added.

[1] Received for publication Oct. 13, 1930; issued April, 1931.
[2] Reference is made by number (italic) to Literature Cited, p. 431.

The whole hegari grain was obtained from the University of Arizona experiment farm and ground to a flour. Whole yellow corn was secured from Kansas and likewise finely ground.

High-grade alfalfa-leaf meal containing 20 to 22 per cent protein was obtained from a local milling company. The casein was a commercial unextracted product. The dried, powdered yeast used was obtained from the Northwestern Yeast Co.

Vitamin D was provided by the incorporation of 0.5 gm. of cholesterol from 1,000 gms. of food which had been irradiated for 30 minutes at a distance of 2 feet from an ultra-violet lamp of the Hanovia mercury-vapor quartz type.

Litter mates, matched as to size and sex as far as possible, were placed upon the different experimental rations. The living conditions of all the animals were identical, the rats being reared in square metal cages with false screen bottoms which were changed daily. Not more than six animals were kept in one cage. The experimental rations and distilled water were fed ad libitum. Weekly records of the weights of all the animals were kept throughout the duration of the experiment, and for a limited time the food intakes were measured and compared.

All of the rations were palatable to the animals, although a greater tendency to scatter the rations containing the higher percentages of alfalfa-leaf meal was noted. The food intake per gram of gain in body weight increased somewhat as the percentage of alfalfa-leaf meal in the ration was increased because of the lower caloric value of these rations.

Males and females were housed together at all times, and the age of animals at mating and the frequency of their matings were not controlled. Pregnant females were separated from the lot cages at least one week before parturition and were weighed daily until after the young were delivered. The nesting material provided the mothers after their young were born consisted of finely cut pure cellulose. The size of the litter was not restricted, each mother being allowed to suckle all her young.

The young animals were weighed as a litter weekly until they were 4 weeks of age, at which time they were weighed separately, numbered, and recorded as individuals, and the mother was returned to the lot from which she came.

Litter mates, matched as to size and sex as far as possible, were placed upon the different experimental rations. Three males and three females were placed upon each ration at the beginning of the experiment, and more animals were started upon these same diets, with the exception of diet 38, from time to time as they became available. A greater number of animals were placed upon ration No. 37 because this ration early showed signs of inadequacy, and further evidence of this kind seemed desirable. In every case a representative pair of young rats from the first litter of each of the three original females, and as far as possible from all females on each ration, was continued on the diet of the parent animals. In this way a study of the effect of the ration on four generations was made.

Criteria for judging the adequacy of each experimental ration consisted in such factors as have been emphasized by Sherman (*4*) to be related to optimum health, namely, rapidity of growth, maximum size obtained, age of maturity, success of reproduction and rearing of

the young, and physical vigor of parents and offspring, including the duration of their prime of life.

That vitamin A can be stored in the animal body and that the amount stored depends upon the concentration of vitamin A in the ration upon which the animal feeds has been proved conclusively by Sherman and his coworkers (*3*, *5*, *7*). Thus, young animals may have at weaning a considerable store of vitamin A, the amount depending upon the concentration of vitamin A in the mother's diet. Such a store is a nutritional asset of great importance in building up reserve resistance to infection, etc. Within limits, then, a ration to be considered adequate should provide opportunity for storage of this vitamin.

Further to check the adequacy of vitamin A in each of the experimental diets, the opportunity which each ration provided for storage of vitamin A was measured by determining the survival period of young rats, taken at the time of weaning, from mothers reared upon the hegari-alfalfa rations and placed on a vitamin-A-free ration composed of extracted casein 18 per cent, Osborne and Mendel's salt mixture 4 per cent, dried brewer's yeast 10 per cent, cornstarch 67 per cent, and sodium chloride 1 per cent, with 0.5 gram irradiated cholesterol added to each 1,000 grams of the diet. A representative pair of young rats taken from each litter was used in this way. Health observations were made, and a record was kept as to the time of appearance of symptoms of vitamin A deficiency, such as cessation of growth, ophthalmia, etc., which indicated exhaustion of body stores of this vitamin. All the animals were autopsied, and the survival periods of those rare animals showing neither premortem nor post-mortem indications of vitamin A lack were not included in the results as tabulated.

EXPERIMENTAL DATA

GROWTH RECORDS

In Table 2 appear the growth records of male rats reared upon the experimental hegari-grain rations containing different percentages of alfalfa-leaf meal as a supplementary source of vitamin A (rations 38, 39, 40, 41, and 42). These are compared with the rates of gain for a similar period of time of male rats dependent upon hegari (ration 37) or yellow corn (ration 3) for all of their vitamin A and also with the growth performances of males receiving a liberal additional supply of vitamin A in the form of 2 per cent cod-liver oil (ration 4). The tabulated results are shown graphically in Figure 1.

TABLE 2.—*Growth records of male rats on all experimental diets*

FIRST GENERATION

No. and composition of ration	Rats	Average weight at weaning	Average weight at 16 weeks	Average weight at 24 weeks
	Number	*Grams*	*Grams*	*Grams*
3, yellow corn, no alfalfa	6	59	288	343
37, hegari, no alfalfa	12	57	263	305
38, hegari and 1 per cent alfalfa	3	51	276	327
39, hegari and 5 per cent alfalfa	7	56	300	350
40, hegari and 10 per cent alfalfa	6	59	304	357
41, hegari and 15 per cent alfalfa	5	58	304	363
42, hegari and 25 per cent alfalfa	6	53	283	329
4, hegari and 2 per cent cod-liver oil	5	56	325	368

TABLE 2.—*Growth records of male rats on all experimental diets*—Continued

No. and composition of ration	Rats	Average weight at weaning	Average weight at 16 weeks	Average weight at 24 weeks
	Number	*Grams*	*Grams*	*Grams*
SECOND GENERATION				
3, yellow corn, no alfalfa	5	53	260	295
37, hegari, no alfalfa	12	35	219	256
38,* hegari and 1 per cent alfalfa	3	51	282	----
39, hegari and 5 per cent alfalfa	5	59	294	358
40, hegari and 10 per cent alfalfa	5	57	310	369
41, hegari and 15 per cent alfalfa	3	53	290	347
42, hegari and 25 per cent alfalfa	6	48	282	339
4, hegari and 2 per cent cod-liver oil	4	57	334	380
THIRD GENERATION				
3, yellow corn, no alfalfa	3	55	256	286
37, hegari, no alfalfa	4	42	198	237
39, hegari and 5 per cent alfalfa	4	49	290	342
40, hegari and 10 per cent alfalfa	5	52	296	356
41, hegari and 15 per cent alfalfa	3	63	294	363
42, hegari and 25 per cent alfalfa	6	54	304	373
4, hegari and 2 per cent cod-liver oil	4	58	291	360
FOURTH GENERATION				
3, yellow corn, no alfalfa	3	55	259	286
37, hegari, no alfalfa	1	51	218	234
39, hegari and 5 per cent alfalfa	3	57	300	361
40, hegari and 10 per cent alfalfa	3	51	286	342
41, hegari and 15 per cent alfalfa	3	47	280	343
42, hegari and 25 per cent alfalfa	4	48	290	334
4, hegari and 2 per cent cod-liver oil	3	53	308	362

* Ration No. 38 was discontinued in second generation.

Table 2 shows conclusively, as has been previously noted (*8*), that hegari as the sole source of vitamin A in a ration believed to be other-

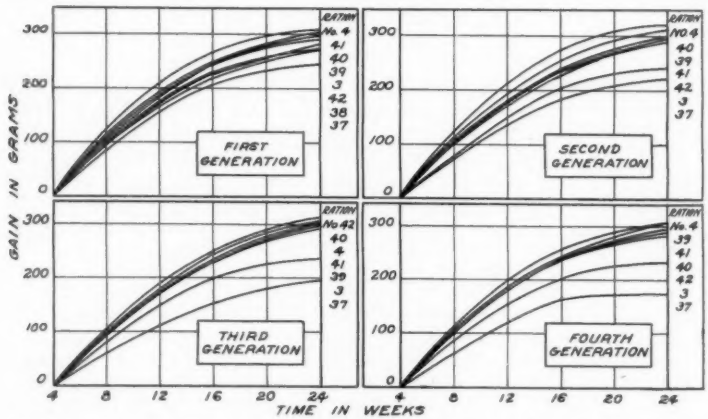

FIGURE 1.—Average growth records of male rats reared on hegari-grain rations containing varying percentage of alfalfa-leaf meal as a supplementary source of vitamin A. Five per cent of alfalfa-leaf meal (ration No. 39) apparently provides sufficient vitamin A to support a normal rate of growth. Increasing the amount of alfalfa meal to 10, 15, and 25 per cent (rations No. 40, 41, and 42, respectively) resulted in no significant differences in growth rate

wise adequate does not provide for an optimum rate of growth of male rats. This inability of hegari to supply sufficient vitamin A for young, growing animals becomes more apparent in each succeeding

generation, as evidenced by a growth rate progressively more retarded in the later generations.

Enriching the vitamin A value of the ration by the incorporation of alfalfa-leaf meal largely compensates for this deficiency of the grain sorghum itself and makes possible a rate of gain more closely approximating the optimum. Whereas 1 per cent of alfalfa-leaf meal resulted in a marked stimulus to growth, the 5 per cent level of feeding gave superior results. Further increases of the vitamin A content of the ration by the use of greater percentages of alfalfa-leaf meal resulted in no greater growth, no significant differences in the growth of animals receiving 5, 10, or 15 per cent of alfalfa meal being observed. In other words the greatest growth rates which were obtained by the use of alfalfa-leaf meal as a supplement to the sorghum grain, hegari, therefore, resulted upon the inclusion of 5 per cent of this material rich in vitamin A. The slightly inferior rate of growth of the males receiving the highest percentages of alfalfa meal was believed to be due to the extensive amount of roughage furnished by the alfalfa. Cases of diarrhea among the animals receiving 15 and 25 per cent of alfalfa meal were common. Just what effect the relativley high fiber content of the alfalfa had upon the assimilation of vitamin A can not be stated.

The somewhat greater weight in most cases of the males reared upon the hegari ration supplemented with cod liver oil as well as the poorer results obtained in the later generations with the yellow corn ration suggest a possible dietary deficiency other than vitamin A. Inadequate vitamin D in a grain ration not sufficiently fortified with calcium is suspected, such a deficiency being remedied by the vitamin D in cod-liver oil, or the calcium and vitamin D in alfalfa, but remaining as a possible limiting factor of the unsupplemented hegari and yellow-corn rations. That vitamin A is the first limiting factor in hegari ration No. 37, which is remedied by the addition of alfalfa or cod-liver oil, is made evident by the data presented later in this paper relative to the comparative stores of vitamin A in the bodies of young rats reared on the hegari and the alfalfa-supplemented hegari rations.

REPRODUCTION RECORDS

Evans and Bishop (*2*) have shown that faulty diet resulting in undernutrition may prevent or postpone the attainment of sexual maturity and greatly affect the ovulation rhythms of female animals reared upon inadequate rations. Observations of the age of rupture of the vaginal membrane of females reared on the experimental hegari and hegari-alfalfa meal rations showed no consistent differences in the age of attainment of sexual maturity of these females that could be correlated with their diet, and no definite abnormality of cycle was noted. The vaginal membrane ruptured in the majority of cases about the fifty-fifth day even in females reared upon ration No. 37, which was low in vitamin A.

Daily microscopic examination of the vaginal smears of many of the females over a limited period of four months, however, showed a strikingly definite abnormality of the ovulation rythm of the females dependent upon hegari as their only source of vitamin A. A disturbance, which has been pointed out by Evans and Bishop (*2*) as highly characteristic of lack of vitamin A, was observed in 100 per cent of the females raised on ration No. 37, namely, a prolongation of the oestrual

desquamative change in the vaginal epithelium. For the entire period of observation in most of the females, the vaginal smear consisted chiefly of the cornified cells, which normally characterize the actual period of ovulation only. Both epithelial cells and leucocytes appeared intermittently, but cornified cells were always present in abundance. Ovulation took place, although irregularly, and a few litters were born to these females.

No similar abnormality of oestrus was observed in the same number of females reared on the grain rations in which alfalfa-leaf meal supplemented the vitamin A in hegari by furnishing additional vitamin A.

Sherman and MacLeod (*6*) have shown that—

> A proportion of vitamin A in the food sufficient to support normal growth may still be insufficient for the added nutritive demand of successful reproduction and lactation.

Reproduction records for the first year of life (approximately one-third of the normal life span) of females reared upon the different hegari-alfalfa experimental rations appear in Table 3.

TABLE 3.—*First-year reproduction records of first-generation females reared upon ration Nos. 37, 38, 39, 40, 41, 42, 3, and 4*

Ration No.	Females used	Average litters per female	Average size of litters	Average young per female	Average young weaned per female		Average weight in grams of young at weaning (28 days)	
	Number	*Number*	*Number*	*Number*	*Number*	*Per cent*	♂	♀
37	12	2.8	5.2	14.6	4.8	32.8	39.0	35.8
38	3	5	6.0	30.0	21.9	73.0	49.8	47.3
39	6	5	7.0	35.0	31.3	89.4	61.9	55.5
40	6	5	7.5	37.5	34.2	91.2	61.1	56.5
41	5	5	7.5	37.5	34.0	90.7	56.5	54.0
42	6	5	7.0	35.0	31.5	90.0	52.4	51.0
3	6	5	7.1	35.5	31.6	89.0	54.0	51.4
4	5	5	8.0	40.0	36.3	90.75	58.5	56.0

The supplemental value of alfalfa as an additional source of vitamin A in hegari-grain rations is again evident from the comparative female reproduction records appearing in Table 3.

In the first year of life the average female reared on ration No. 37, in which hegari furnishes all the vitamin A, produced approximately three litters with an average size of 5.2 young but was successful in rearing but 32.8 per cent of her offspring. At weaning time (28 days of age) they were undersized and weaklings with an average weight of 39 gm. for the males and 35.8 gm. for the females. Addition of 1 per cent alfalfa-leaf meal improved the reproductive performance of the females considerably, enabling them to bear larger litters more frequently and to suckle to the weaning age a larger percentage (73 per cent) of their young. However, the results were far from optimum at this low vitamin level (1 per cent of alfalfa-leaf meal). With the inclusion of 5 per cent of alfalfa-leaf meal in the hegari ration, a much more nearly optimum reproductive performance was obtained. Not only did the females produce more young and wean a larger percentage of them, but the young themselves were in better physical condition, as indicated by their general appearance and liveliness and their decidely greater weaning weight.

Increasing the vitamin A value of the ration still further by the use of greater percentages of alfalfa-leaf meal (10, 15, and 25 per cent) did not materially improve the reproduction records of the females

during the first year. However, the period of reproductive life of the females on ration No. 39 is shorter than that of females which received the higher percentages of alfalfa. For example, female No. 496 reared on ration No. 40 continued to be fertile for 196 days after her sister, receiving only 5 per cent of alfalfa-leaf meal, had ceased to bear young.

Figure 2 shows graphically the comparative growth and reproduction performances of representative litter mates reared upon ration No. 37, low in vitamin A, and ration No. 40, in which the inadequate vitamin A value of the hegari is plainly compensated for by the addition of 10 per cent of alfalfa-leaf meal.

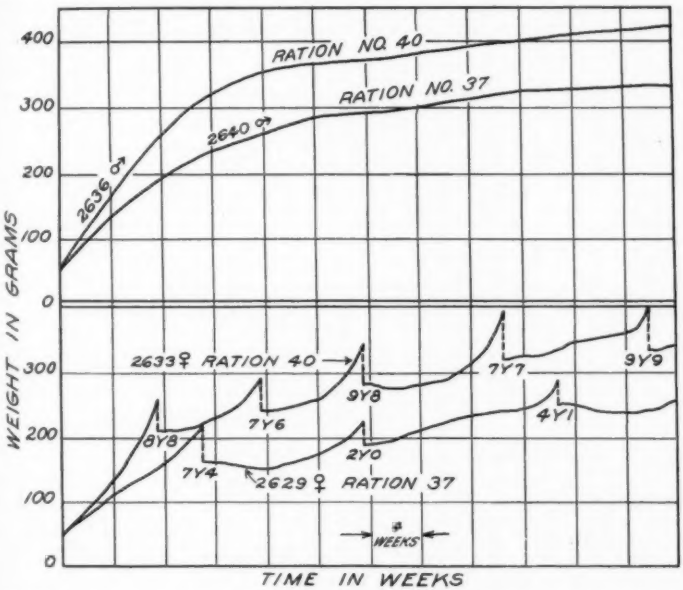

FIGURE 2.—Comparative growth and reproduction records of litter mates reared on hegari-grain rations with and without a supplementary source of vitamin A. Female 2629, reared on ration No 37, bore 13 young in the first year and raised but 5 (38.7 per cent). She died five weeks later with undelivered young. On the other hand, her litter sister ♀ 2633, who had received 10 per cent of alfalfa-leaf meal (ration No. 40), bore 40 young in the same length of time and successfully suckled 95 per cent of them. The break in the female growth curves indicates loss of weight upon delivery of young. The number before the Y refers to the number of young born and that after the Y indicates number of young weaned

STORAGE OF VITAMIN A

The relative stores of vitamin A in the 28-day-old young of females reared upon the sorghum grain alfalfa rations as measured by determination of their survival time when transferred to a ration free from all vitamin A are presented in Table 4 and shown graphically in Figure 3.

That the young which do live to be weaned on ration No. 37 start out in life with but a very small reserve supply of vitamin A and are therefore poorly equipped to withstand infection is shown by the fact that within a week ophthalmia appears, a maximum gain of only 11 gm. is reached, and the animals survive on the vitamin-A-free

ration only 22.6 days. On the other hand, the young of females that have been reared on rations Nos. 38, 39, 40, 41, and 42, containing 1, 5, 10, 15, and 25 per cent, respectively, of alfalfa-leaf meal as a supplementary source of vitamin A possess greater bodily stores of this vitamin at weaning and are better able to withstand subsequent deprivation. It is quite apparent, too, that the increasing percentage

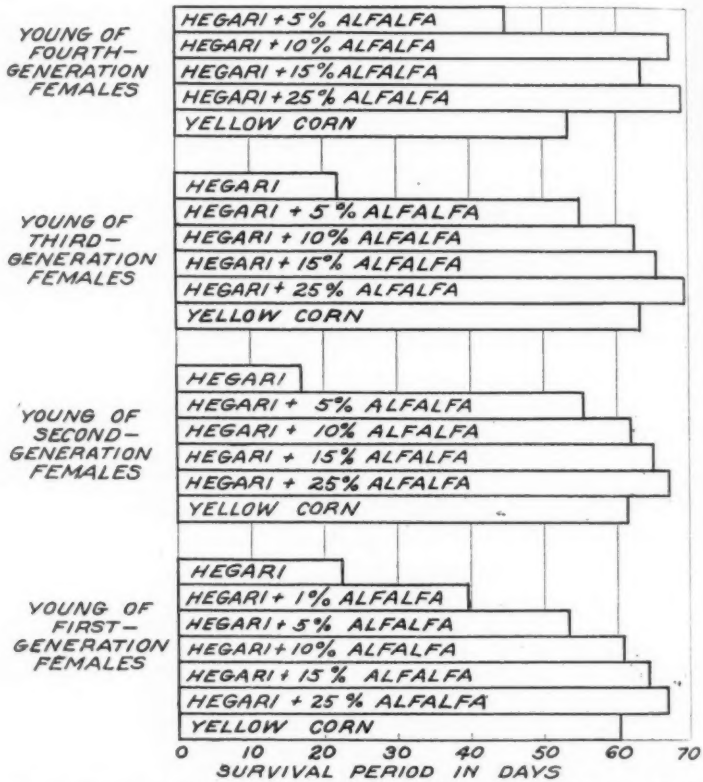

FIGURE 3.—Increase of stored vitamin A in rats as measured by the survival period upon a vitamin-A-free diet, when increasing percentages of alfalfa are fed in the ration. The increment of increase becomes progressively smaller with each increasing percentage of alfalfa leaf meal fed

of alfalfa-leaf meal in the ration is reflected directly in the increasingly greater bodily store of vitamin A in the young at weaning for a prolongation of the survival period (from 22.6 to 67 days), a greater delay in the appearance of the signs of infection which characterize the lack of vitamin A, and the attainment of a larger maximum weight before decline set in, accompanied the inclusion in the ration of increasing percentages of alfalfa.

TABLE 4.—*Storage of vitamin A in 28-day young of females reared on experimental rations as shown by their survival on vitamin-A-free diet*

Ration No. and composition	Mothers represented	28-day-old offspring placed on vitamin-A-free diet	Average survival on vitamin-A-free diet
	Number	*Number*	*Days*
YOUNG OF FIRST-GENERATION FEMALES			
37, hegari, no alfalfa	12	43	22.6
38, hegari and 1 per cent alfalfa	3	16	39.6
39, hegari and 5 per cent alfalfa	6	36	53.5
40, hegari and 10 per cent alfalfa	6	40	61.0
41, hegari and 15 per cent alfalfa	5	32	64.5
42, hegari and 25 per cent alfalfa	6	40	67.0
3, yellow corn	6	42	60.3
YOUNG OF SECOND-GENERATION FEMALES			
37, hegari, no alfalfa	[a] 2	4	17.0
39, hegari and 5 per cent alfalfa	4	22	55.4
40, hegari and 10 per cent alfalfa	6	22	62.0
41, hegari and 15 per cent alfalfa	5	18	65.0
42, hegari and 25 per cent alfalfa	6	25	67.3
3, yellow corn	4	20	61.8
YOUNG OF THIRD-GENERATION FEMALES			
37, hegari, no alfalfa	2	2	22.0
39, hegari and 5 per cent alfalfa	3	14	55.0
40, hegari and 10 per cent alfalfa	4	24	62.6
41, hegari and 15 per cent alfalfa	3	20	65.8
42, hegari and 25 per cent alfalfa	4	22	69.7
3, yellow corn	4	16	63.1
YOUNG OF FOURTH-GENERATION FEMALES			
37, hegari, no alfalfa	[b]		
39, hegari and 5 per cent alfalfa	3	12	45.0
40, hegari and 10 per cent alfalfa	4	14	67.6
41, hegari and 15 per cent alfalfa	3	12	63.4
42, hegari and 25 per cent alfalfa	4	18	69.2
3, yellow corn	4	16	53.5

[a] Only 2 out of 9 second-generation females on ration No. 37 raised young.
[b] No young raised by the 1 fourth-generation female on ration No. 37.

However, it may be noted here, as has been previously shown by Sherman and his coworkers (*3*, *6*), that the store of vitamin A does not increase in arithmetical proportion to the concentration of this vitamin in the ration. (Fig. 3.) The animals appear to use the vitamin less economically as its concentration in the food is increased. Thus enriching the vitamin A value of the rations with 1 per cent of the supplement, alfalfa-leaf meal has made possible an increase in storage of 77 per cent over that in animals which have been dependent upon hegari for all their vitamin A (ration No. 37). An increase in storage of vitamin A of approximately 137, 170, 185, and 196 per cent was found in rations containing 5, 10, 15, and 25 per cent, respectively, of alfalfa-leaf meal. It may be seen that the increment of increase in the store becomes smaller as the concentration of vitamin A in the rations is increased by the use of the greater percentages of alfalfa.

Whereas 1 per cent of alfalfa increased the store of vitamin A in the bodies of the young rats by 75.2 per cent over that in the animals on ration No. 37, the addition of 4 per cent more resulted in a further increase of 35.1 per cent, the next 5 per cent (ration No. 40) increased the store 14 per cent more, whereas the next additional 5 per cent gave a further increase of but 5.7 per cent, and the final increase of 10 per cent of alfalfa increased the amount of the reserve supply by only 3.8 per cent, which shows a decrease in efficiency of storage of the vitamin as the basal ration was enriched.

LONGEVITY RECORDS

A definite tendency toward longer life as the diet is made richer in vitamin A has been demonstrated by Sherman and Campbell (*4*) and again by Batchelder (*1*). Although longevity records of the animals on all of the experimental hegari-alfalfa rations are incomplete, the data at hand give further proof of the inadequacy of hegari as a source of vitamin A and of the supplemental value of alfalfa-leaf meal.

At the time of the present writing more than two years (849 days) have elapsed since the first-generation animals were transferred from the stock colony to the experimental rations. In this period only one animal on a hegari ration supplemented with alfalfa has died and this was a female who developed a large tumor weighing 500 gm. after having shown an excellent reproduction record.

On the other hand, of the 22 first-generation animals reared upon ration No. 37 in which hegari serves as the only source of vitamin A, with the exception of one female who has never raised a litter and is still living, all of the animals have died after a relatively short life span (552 days average for the males and 321 days for the females). Most of the females on this ration were pregnant at the time of death, dying during parturition. Autopsy records revealed undelivered young. The second-generation animals being less fortified with vitamin A at birth, and therefore less thrifty than their parent animals taken from the stock colony, exhibited a shorter life span (287 days average for the males and 213 days for the females). Only six third-generation, and two fourth-generation rats were raised on this ration, the families therefore practically dying out in the third generation.

Although all the animals, save one, on the rations containing alfalfa-leaf meal are still living, a great difference in vigor and in their outward appearance may be noted. Animals on ration No. 39 containing 5 per cent of the alfalfa-leaf meal show signs of old age, thin unkempt hair, lack of vitality, and are losing weight, whereas the animals of the same age which have received 10 or 15 per cent of alfalfa are in much better physical condition, and better appearing in every way. It would seem that 5 per cent of alfalfa-leaf meal enabled the young males to grow at a normal rate and the females to reproduce successfully for a time at least, but the drain upon the mother animal is excessive, the reproductive period is shorter, and the animals pass their prime of life at a relatively earlier age and in all probability will show a shorter life span.

SUMMARY AND CONCLUSIONS

In order to determine the supplementary value of alfalfa-leaf meal as a food material rich in vitamin A for use in sorghum-grain rations, albino rats of the same nutritional history, matched as to sex, weight, and litter, were placed upon hegari (a sorghum grain) rations containing alfalfa-leaf meal ranging in amount from 1 to 25 per cent (ration Nos. 38 to 42, inclusive). The amount of alfalfa-leaf meal which should be incorporated into the ration to compensate for the lack of vitamin A in the hegari itself was indicated by such criteria as the ability of the ration to support an optimum rate of growth in young male animals, to permit females to reproduce and successfully suckle many vigorous offspring, to provide opportunity for storage of sufficient vitamin A to increase the body ability to resist infection, and to enable the animals to live to a healthy old age.

Growth records show that 5 per cent of alfalfa-leaf meal provides sufficient vitamin A to promote good growth. No better growth was obtained with the higher percentages of alfalfa leaf meal.

Reproduction records show that females receiving 5 per cent of alfalfa-leaf meal produce healthy, thrifty young and are successful in suckling a large percentage of them. However, 10 per cent of alfalfa-leaf meal insures a longer reproductive life, the females on ration No. 40 raising many young after the reproductive life of females on ration No. 39 is passed.

A measure of the relative stores of vitamin A in the bodies of the young at weaning time shows an increasing store as the percentage of alfalfa-leaf meal is increased. The increment of increase in store with each percentage increase in the ration of the vitamin-A-rich alfalfa becomes decidedly less when more than 10 per cent is incorporated in the ration.

Longevity records, although incomplete, show that whereas 5 per cent of alfalfa-leaf meal results in striking prolongation of life, when 10 per cent is incorporated in the ration the animals show signs of old age at a still later date and will in all probability live longer.

Taking all points into consideration, it would appear that whereas incorporation of 5 per cent of alfalfa-leaf meal in the sorghum-grain ration largely compensates for the lack of vitamin A in the hegari itself resulting in a striking stimulus to growth, improving markedly the female reproductive performances, etc., the use of 10 per cent of alfalfa-leaf meal produces more nearly optimum results.

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URINARY CALCULI IN SHEEP[1]

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INTRODUCTION

The occurrence of calculi in sheep may not be more frequent than in other animals, but present knowledge of the subject indicates that it probably is. Possibly this trouble occurs much more often than is generally supposed, for if the calculi are small and few in number they may not noticeably affect the animal and therefore may escape detection. Furthermore, it is highly probable that a good many cases are missed because the average sheepman and some veterinarians do not recognize the symptoms.

Frequent references in early livestock literature to "gravel" and "stones," the terms most commonly used by sheepmen to designate calculi, indicate that they have long been known and recognized by experienced sheepmen. The cause of their formation has been attributed to (1) roots, particularly mangels; (2) hard water; (3) high condition; (4) lack of exercise; and (5) chilling from east winds in the early spring after shearing. When sheep are affected with calculi, the voiding of urine is restricted and, in some cases, is entirely prevented. Concerning this symptom, Youatt (*14, p. 588–589*)[3] says:

> It is mostly seen in males, and particularly rams, while being got up for exhibition purposes, under the influence of rich highly stimulating food.
>
> The chief cause of this defect as seen in sheep is due to the presence of minute sandy particles, which block up the urinary passage at the extreme end of the penis (worm). This gravelly matter is deposited from the urine while in the bladder, and when passing along the canal becomes arrested in the small worm-like appendix of the organ, thus shutting in the urine and preventing its escape. The deposition of sandy matter in the bladder is said by practical flock-masters to arise from the excessive use of mangels and other foods rich in sugar. We think, however, that the want of exercise and the habit of going for long periods without emptying the bladder, as do fat lazy sheep, has much to do with it.

A survey of the literature indicates that the calculi are usually produced by formations of calcium carbonate, calcium and aluminum phosphates, aluminum silicate together with kidney tissue urates, epithelium, etc. Michael (*10*) studied calculi of sheep for five years. He obtained data from lots of rams fed (1) grain and clover hay; (2) grain, hay, and corn silage; (3) grain, hay, and mangels; and (4) grain, hay, and sugar beets. In lots 1 and 2 no calculi were found, and the urinary organs were normal, but in lots 3 and 4 there were a number of cases of calculi, and all but four of the rams in lot 4 had distended bladders. Six of the 11 in lot 4 had enlarged gall bladders, 4 had enlarged hearts, 6 had enlarged kidneys, and most of them showed irritation in one or more parts of the urinary tract. Michael

[1] Received for publication Aug. 8, 1930; issued April, 1931.
[2] The writers are indebted to Samuel Breese, shepherd of the Purdue flocks who fed the experimental rations and assisted in obtaining the urine samples and in diagnosing the calculi cases; to Dr. J. R. Mohler, Dr. G. F. Creech, D. A. Spencer, of the United States Department of Agriculture, and Prof. Claud Harper for obtaining certain data on the lambs used in the experiments; to Elizabeth Heiss for the drawing shown as Figure 1; and to Dr. E. J. Kohl for making the photographs.
[3] Reference is made by number (italic) to Literature Cited, p. 446.

attributes the enlargement of the organs to the large amount of water ingested, which caused distention and increased the activity of the kidneys. These results would appear to indicate that the mangels and sugar beets were responsible for the formation of calculi.

Hager and Magath (*4, 5, 6*) produced vesical calculi experimentally in rabbits and guinea pigs. They catheterized the bladder and then introduced 0.5 c. c. of a 0.1 per cent alcoholic solution of salicylic acid, which produced a marked excoriation of the mucosa. After this injection, 1 c. c. of a 24-hour broth culture of *Proteus ammonieae* was introduced into the bladder through a catheter. This resulted in the production of a severe grade of incrusted cystitis which lasted for an indefinite period, the urine becoming markedly alkaline. These investigators recognized bacteria from two sources which they believe are concerned in "stone" formation; (1) endogenous, which originate in foci in the body, and (2) exogenous, which gain entrance to the urinary tract by direct implantation. Post-mortem examinations revealed both free stones and incrustrations. Their conclusions (*6, p. 269*) in part are that—

> Proteus ammonieae, being able to convert urea rapidly into ammonia, precipitated the alkaline earthy salts in the urine, forming chiefly the carbonates and phosphates of calcium and magnesium; these precipitates became attached to the abraided mucous surface. It was noted at the time of the experiments that there was a tendency for these precipitates to break off and form free calculi in the bladder. * * *

Several different investigators have obtained results which indicate that certain dietary deficiencies may be a predisposing cause of calculi. Osborne and Mendel (*11*) found calcium phosphate calculi prevalent in rats which had been kept on a diet deficient in vitamin A. A study of the diet of these rats showed that "in every instance where calculi developed, the animals were without an adequate source of the fat-soluble vitamin for some time." Fujimaki (*3*) found that rats fed a diet deficient in vitamin A developed bladder calculi, but when vitamin A was again included in the diet the bladder stones disappeared. Animals fed on a diet deficient in vitamin A, inorganic phosphorus, and calcium formed calculi in a very short time. Bile duct stones took longest to form, and the urinary calculi formed were carbonates. Calculi in animals fed diets deficient in vitamin A or vitamins A and C were phosphatic. McCarrison (*8*) obtained vesical calculi in rats fed diets characterized by the absence of protein of animal origin, a deficiency of vitamin A, and an excess of earthy phosphates. Stones were found in 29 per cent of the 72 animals used in the experiment, but the stones did not appear until the unbalanced diet had been fed for 56 days. Keyser (*7*) found that feeding oxamide produced artificial concretions consistently. Oxamide is a foreign crystalloid in the urinary tract. It is precipitated with colloidal material of urine in such a way that fusion of crystals occurs. Concretions are apparently of endogenous origin and are due to defects in metabolism which cause changes in the hydrogen-ion concentration or colloidal content of the urine.

The occurrence of xanthine calculi in sheep has been reported by Easterfield, Rigg, Askew, and Bruce (*1*). This type is widespread among sheep on poor soils in New Zealand, especially on soils that are deficient in limestone and phosphoric compounds. The greatest incidence of calculus occurred where great deterioration in pasture

had taken place. The pasture grass is very high in nitrogen compounds, the nitrogen ranging from 3.43 to 5.23 per cent. Sheep grown on this pasture are badly stunted, and some of the excess nitrogen in the bladder is deposited in the form of xanthine ($C_5H_4N_4O_2$). Where the poor soils were limed and treated with a phosphate fertilizer and good pastures established, no calculi in the kidneys of sheep were recorded.

Another cause of calculi formation was reported by Tennant (*13*). He found instances in which the sulphur contained in food was not oxidized and eliminated as sulphates but as cystine. This amino acid, being very insoluble in urine, formed human kidney stone concretions.

The results of Evvard, Culbertson, and Wallace (*2, p. 585*) indicate that molasses in the diet has some effect on the formation of urinary calculi in feeding lambs. Lots fed beet molasses showed a larger percentage of cases than lots fed cane molasses. Thirty per cent and 48 per cent, respectively, of the lambs in lots fed daily 0.5 pound and 0.7 pound of molasses per lamb had calculi. Evvard et al. believe that "these calculi findings are of practical significance inasmuch as they show quite unmistakably that the feed is a factor in their formation."

FREQUENCY OF OCCURRENCE OF CALCULI IN SHEEP

An attempt was made to obtain data on the prevalence of urinary calculi in sheep. For this purpose a questionnaire was sent to 53 prominent sheepmen in different parts of the United States and southeastern Canada. Forty-five replied. Of these only eight had had no calculi trouble in their flocks and did not know of its occurrence. The eight replies came from New Hampshire, New York, Vermont, Massachusetts, Ohio, Virginia, West Virginia, and Texas. However, other replies from four of these States reported cases of calculi. In all, 230 cases, covering a period of from 2 to 15 years, were reported by 40 different men. Twice as many cases of calculi were reported where roots were not fed as where they were. Several instances were mentioned in which rams were fed a ration composed chiefly of roots for several months without any trouble from calculi. Apparently, drinking soft water does not always prevent the formation of calculi, as several cases were reported in sheep that had had only soft water to drink. The occurrence of the trouble was said to be most frequent during the winter months when the ration contained a considerable amount of grain, or when the sheep were being fed heavily on grain for exhibition purposes. However, cases were reported which occurred during the summer when the sheep were on pasture and were being fed grain, and in a few instances, when they were on pasture and received no grain. The rations fed to the sheep affected were composed, in most cases, of the following feeds: Corn, barley, oats, bran, linseed meal, legume hay, and corn silage.

Some sheepmen believe that this trouble is restricted largely to limestone sections. The present writers are convinced that this trouble is not restricted to such regions for they have records of cases which have occurred in regions in which the soil is quite acid. As far as one may judge from these reports it appears that the occurrence of calculi is rare in the New England States, New York, Maryland,

Pennsylvania, Virginia and West Virginia, and that it is relatively common in Indiana, Wisconsin, Iowa, Kansas, Missouri, Oregon, Utah, Colorado, and California.

OBSERVED CASES OF CALCULI

During the past five years, 12 well-defined cases of calculi have been observed in the Purdue purebred flock, and detailed records of these have been kept. Fifteen western feeding lambs which were brought to the veterinary clinic of the station for post-mortem diagnosis had calculi. They came from four different farms. During the winter of 1929–30, 15 cases were observed among the 224 western feeding lambs being fed experimentally at the Purdue experiment station. Five lambs died from calculi during the feeding period and the remaining 219 were posted when slaughtered. Ten had calculi and in 67 others evidence was found of irritation in the urinary tract which strongly

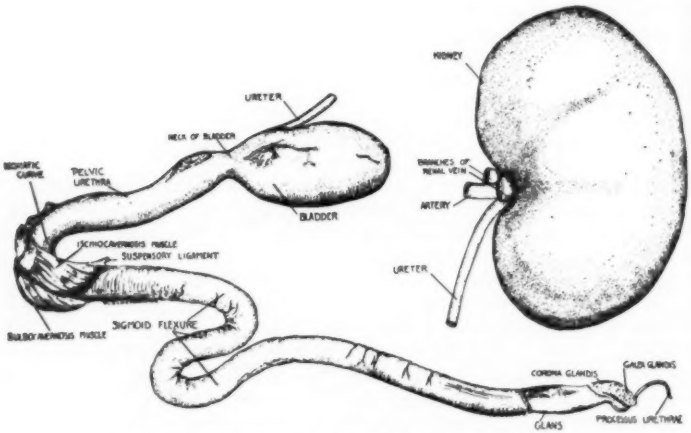

FIGURE 1.—Urinary tract of the male sheep. (After Sisson) (*12*)

suggested either that calculi had been present and had passed out with the urine or that the conditions which prevailed in the urinary tract were conducive to the formation of calculi. However, this was the first time that any appreciable amount of this trouble had been observed in feeding lambs at this station during 17 years of sheep-feeding trials.

The anatomical features of the urinary tract in the male sheep favor the lodgment of calculi or other obstructive bodies in the urethral portion of the tract. There are three places in the penial urethra at which calculi are particularly likely to lodge, namely, the ischial curve, the sigmoid curvature, and the processus urethrae or "worm." The changes in direction and the accompanying narrowing of the lumen of the urethra at the curvatures make it difficult for calculi to pass these points. The small lumen of the processus urethrae is likely to cause the retention of calculi of any appreciable size. (Fig. 1.)

Only a few typical cases observed by the writers will be described. The details vary considerably in individual cases but the major fea-

tures are common to all. The writers have found calculi in every part of the urinary tract, but in only one case were calculi found in every part of the urinary tract in the same sheep.

CASE No. 1 (Purdue No. 1664).—A Rambouillet yearling ram had an attack of calculi in May, 1928, which was a "dry" case. He was operated on but without success and was immediately killed and posted. Calculi were found in both kidneys as well as in the bladder, and the ureters and urethra were nearly filled with small concretions throughout their entire length. Stoppage was complete in both the ischiatic and sigmoid curvatures. The bladder and kidneys were greatly distended with urine, the kidneys being three times their normal size. There was necrosis in the bladder wall extending over an area about 4 cm. in diameter. Rupture of the bladder at this point would evidently have occurred within a few hours. The ram had been fed a mixture of corn and oats, alfalfa hay, and silage during the winter, but had been on pasture for 10 days previous to the attack. He was a rugged, healthy sheep and was not in high condition when stricken.

CASE No. 2 (Purdue 1498).—A 3-year-old Rambouillet ram had an attack on February 24, 1928. When he was first observed, stoppage was only partial, but two days later became complete. An examination of his "worm" was made, and a calculus was found in it. (Fig. 3.) The worm was severed and voiding of urine began immediately. In a few days, recovery was complete.

CASE No. 3 (Purdue 1698).—A 2-year-old Shropshire wether had his first observed attack October 17, 1929. It was not severe, and in three days he appeared to have recovered. On October 23, 1929, he had a second attack which was more severe than the first. The voiding of urine was reduced to a slow seepage. His worm was examined and found to be normal. A probe was inserted into the urethra as far as the first part of the S curvature, but without result. It was decided that the obstruction was so located that there was no hope of removing it. Consequently, the animal was killed and posted. Only one calculus was found in the entire urinary tract. It had lodged in the proximal portion of the sigmoid curvature. During the summer, and until stricken, the wether had run with the ewe flock on pasture; and for several weeks previous to the attack he had been fed one-half pound daily of corn and oats, equal parts.

CASE No. 4 (U. S. D. A. 59 C).—A 3-year-old Southdown ram had his first attack January 1, 1927. Examination revealed small calculi in the worm, but removal of the worm gave only temporary relief. Further examination showed that calculi had lodged in the urethra below the sigmoid curvature. They were located by forcing the penis out of the sheath and pressing the penis between the thumb and first finger. The calculi were forced out of the penis. Voiding of urine began and the ram improved steadily for three days. On the fourth day, however, he showed marked symptoms of pain and 24 hours later died. A post-mortem examination showed a gangrenous condition of the penis, which was evidently caused by forcing the concretions out of the urethra No calculi were found in the other parts of the urinary tract.

CASE No. 5 (Purdue 1750).—A Hampshire yearling wether showed the first symptoms of calculi July 5, 1929. A large calculus was found about 1 inch above the proximal end of the worm. The worm was inflamed but had not adhered to the penis. It was severed and the calculus was removed. The wether's condition improved, but three days later a second attack occurred in which urination was inhibited. Several doses of formin tablets were administered and the animal recovered. It is by no means certain, of course, that the formin was responsible for his recovery. He had been fed a grain ration of 3 parts oats, 1 part corn since January 1 and had been on rape and bluegrass pasture for three weeks previous to the attack.

AUTOPSY REPORT

When the voiding of urine is largely or wholly inhibited for a period of about 48 hours, the bladder or some other portion of the urinary tract usually ruptures. About 24 hours later death occurs. The conditions found in an autopsy of a ram which had calculi, causing the bladder to rupture, are presented below.

The abdominal cavity contained a large amount of bloody urine. There was also a slight amount of fibrinous peritonitis. The scrotum

contained bloody fluid and some clots of fibrin. The fat in the region of the kidneys and the renal lymph glands was infiltrated with fluid. The gall bladder was distended with bile. No other lesions were found outside the genitourinary organs. The ureters were distended with bloody urine and showed the effects of many hemorrhages, particularly near the kidneys. The kidneys were somewhat enlarged, due mainly to distention of the pelves and calyces by urine. Large hemorrhages had occurred under the lining of the pelves; and the kidney cortex and medulla contained many petechiae. There was some gross evidence of parenchymatous degeneration in the kidneys. The capsules of the kidneys stripped easily. No calculi were found in the ureters or kidneys.

The urinary bladder was perforated in the dorsal wall, and showed many subserous and submucous hemorrhages. There were many whitish, rather soft calculi and some blood clots in the bladder. Some of the calculi were 1 cm. in diameter. The penial urethra was packed with calculi from a point at the beginning of the ischial curvature to a point about an inch beyond the beginning of the S curve. The urethra was free from obstruction throughout the rest of its course, but was hemorrhagic throughout its entire length.

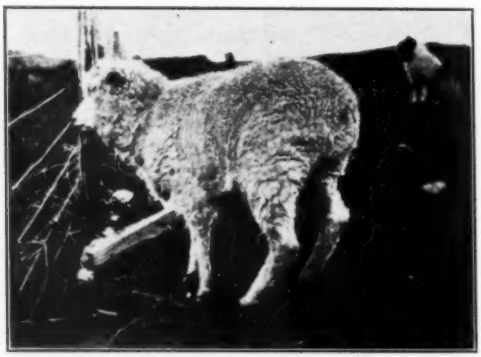

FIGURE 2.—Typical attitude of a wether showing pronounced symptoms of calculi. (From Easterfield, Rigg, Askew, and Bruce (*1*))

SIZE AND COMPOSITION OF CALCULI

The cases studied were of two kinds—those in which one or more large stones were present, and those in which large numbers of small stones were present. The stones varied in size, shape, crystalline structure, hardness, and chemical composition. The large ones had relatively larger deposits of mineral salts and were usually harder than the smaller ones. The small ones looked much like fine grains of sand. They contained relatively more organic matter than the large ones, were porous, and generally soft enough to crumble when pressed between the fingers. The concretions were composed of calcium phosphate or aluminum silicate, largely the former, depending on whether grain or hay was most abundant in the ration.

SYMPTOMS OF CALCULI

Partial or complete stoppage of the urinary tract is the most positive evidence of calculi. Affected sheep refuse their feed, or eat very little of it, the head is often carried low, the back becomes arched, especially when the animal strains in trying to urinate, and there is a tendency not to exercise. (Fig. 2.)

The animals become dull and listless and, in advanced stages, are very sick. They are inclined to lie down most of the time, usually on their bellies. When they get up, they frequently stamp their hind feet. The wool around the end of the sheath is almost invariably wet. If there is any suspicion that a sheep may have stones, the wool immediately around the end of the sheath should be examined. This symptom is frequently the only reliable one in the early stages.

In Figure 3 is shown a worm with a large calculus in it. This specimen was obtained from a Rambouillet ram, Purdue 1498. Stoppage was complete. The stones in the worm can be felt with the fingers and may be removed in some cases by pressure, but it is generally better to sever the worm near its proximal end. In some cases, the worm becomes attached to the penis. This condition was found in the majority of the cases observed, and evidently favored the lodgment of calculi in the "worm."

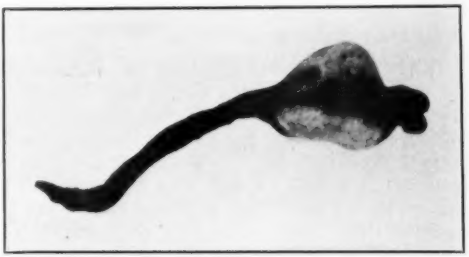

FIGURE 3.—Calculus lodged in the lumen of the processus urethrae (worm) of a Rambouillet ram

RECURRENCE OF ATTACKS

The first attack may or may not prove fatal. If the symptoms are recognized soon enough, and if the stoppage is in the worm, the calculi can be removed and recovery follows, provided there is no stoppage in other parts of the urinary tract. In the cases studied by the writers, those that survived the first attack—and a large percentage did—had a second attack, which was always more severe than the first. The number of recoveries was small, partly for the reason that in the Purdue flock a second attack generally meant that the calculi had lodged above the curvature, since the worm was usually removed at the time of the first attack. A few sheep have survived the second attack, but a third attack has proved fatal in every case. One attack is generally followed by a second attack, and in a few cases by a third. Wether lambs are not kept after they have recovered from the second attack.

PRELIMINARY OBSERVATIONS AND EXPERIMENTS

A survey of the literature showed that published data on the composition and hydrogen-ion concentration of sheep urine are very meager. It was evident, therefore, that it would be necessary to obtain samples of urine from a number of different sheep kept on known rations in order to determine what should constitute normal urine from animals on these rations. This was done, and it was found that the pH values of urine from different individuals ranged from 6.8 to 9.0, depending on the kind of ration fed, and possibly to some extent on

the individuality of the sheep. This finding suggested that a study of the urine might reveal to what extent the ration is utilized in metabolism.

FEEDING EXPERIMENTS

Feeding experiments designed to show how a certain feed affects the reaction or composition of the urine were formulated. For these tests sheep 1 year old and older were selected from the Purdue flocks. Ewes, wethers, and a few rams were used. A crate 4 by 6 feet having a ½-inch mesh-wire bottom and a drain pan was constructed. The mesh-wire bottom was covered with burlap which permitted the collection of both feces and urine. The sheep were fed in dry lots and were prepared for the experiment by being fed the ration to be tested for two weeks prior to the beginning of the experiment.

FIRST SERIES

In the first series of experiments three different rations were selected for comparison. Three sheep were placed in each of three different lots. Lot 1 was fed alfalfa hay only; lot 2, corn silage and grain, two parts oats and one part corn; lot 3, mangels and corn stover. The rations were fed for six weeks, and composite samples of urine and feces were taken during this interval. Two sheep were placed in the crate for each run, and the runs were for 24-hour periods. The average daily feed and water consumption per sheep, the amount of urine voided, its pH concentration, its percentage of total solids and ash, and the amount of 0.20 N hydrochloric acid required to neutralize 100 c. c. of urine ash, are given in Table 1.

TABLE 1.—*Influence of the first three rations tested upon the quantity and composition of the urine voided; average per sheep per day*

Lot No.	Daily feed	Average daily water consumption	Average urine voided daily	pH of urine	Total solids of urine	Ash in urine	Quantity 0.20 N HCl required to neutralize 100 c. c. urine ash
		Pounds	*C. c.*		*Per cent*	*Per cent*	*C. c.*
1	2.87 pounds alfalfa hay	2.79	312	9.0	8.5	5.5	90.6
2	2.6 pounds grain; 1.75 pounds corn silage	1.44	207	7.16	6.7	1.75	.2
3	11 pounds mangels; 0.6 pound corn stover	0	2,310	8.62	1.63	.91	33.2

The data in Table 1 show significant differences in the quantity of urine from the three lots. The large amount voided by the sheep in lot 3 is of particular interest. Twenty-three hundred and ten cubic centimeters per sheep during 24 hours is a very large output. It should be noted that the sheep in this lot drank no water. The differences in the pH values, the percentage of ash, and the amount of 0.20 N HCl required to neutralize the water-soluble bases in the ash, are of especial interest in connection with the problem under consideration.

The urine from the sheep in lot 1 was very alkaline when voided, having a pH value of 9.0 to 9.2. It contained 8.5 per cent of total solids, 5.5 per cent of ash, and required 90.6 c. c. of 0.20 N HCl to neutralize the water-soluble bases in the ash of 100 c. c. of urine. These data indicate that an abnormally large quantity of nutrient

material was being eliminated by way of the kidneys, and the large ash content might suggest a tendency to form calculi, but it was mostly water-soluble and there was no evidence of the formation of any gravel by combination with the potassium or other elements present.

The sheep in lot 2 produced what appeared to be more normal urine, having a pH value of 6.9 to 7.3 with a volume of 225 c. c. per sheep in 24 hours. The total solids averaged 6.7 per cent and the ash 1.75 per cent. That the ash material contained only traces of soluble bases is shown by the fact that only 0.2 c. c. of 0.20 N HCl was required to neutralize 100 c. c. of urine ash. This would indicate that the soluble bases in the feed were neutralized by acid radicals—phosphates, silicates, and sulphates—contained or produced by the feed.

The fresh urine from lot 3 had a pH range of 8.4 to 8.8. It contained only 1.63 per cent of total solids and 0.91 per cent ash. The relatively large amount of 0.20 N HCl required to neutralize the alkalinity of the ash of this very dilute urine appears to the writers to be very significant. It was due largely to the presence of compounds of potassium in relatively large amounts. Since this element does not form many compounds which are insoluble in water, it is not probable that the cause of calculi formation is the presence of beets in the ration, but rather that the beets, so far as they are concerned, may serve to prevent the formation of calculi through the production of water-soluble in place of water-insoluble compounds.

Many cases of calculi have been reported in sheep which were being fed roots, particularly mangels, but the rations fed in these cases almost invariably contained, in addition to the roots, legume hay and grain. Roots may be a contributing factor when fed along with hay and grain, for the calcium in the roots may combine with the phosphorus in the grains to form calcium phosphate which is the chief component of most of the calculi analyzed by the writers. This is an admission that roots may be a contributing factor under certain conditions, but not that roots are a primary cause of calculi formation. The fact that calculi are formed when the ration does not contain roots and when sheep have never been fed roots, leads the writers to believe that their occurrence is not wholly dependent, if at all, on the presence of roots in the ration.

The replies to our questionnaire mentioned showed that its occurrence was more frequent when the ration did not contain roots. The present studies lead us to believe that calculi formation is impossible when roots only are fed, and that they are not likely to be formed when the ration is composed of roots and legume hay, or roots and grain.

The three lots of feeds used in these experiments represented widely different mineral balances. The potassium compounds predominate in the basic material in beets, making the urine ash alkaline, as there are no acid-producing compounds present in quantity with which to combine. The alfalfa hay ration is outstanding in its calcium content but low in phosphorus and sulphur. Hence, the urine ash is very alkaline. The grain and silage ration is low in basic materials and high in silicates and phosphates. Hence, the urine ash is usually neutral or even slightly acid.

SECOND SERIES

In a second series of experiments the study was extended to include a number of different combinations of feeds.

These are shown in Table 2.

TABLE 2.—*Influence of various rations fed upon the quantity and composition of urine voided by sheep*

Ration	Average amount of urine voided	Total solids in urine	Quantity 0.20 N HCl required to neutralize 100 c. c. urine ash	Ash in urine	pH of urine	Crystals formed in standing urine
	C. c.	*Per cent*	*C. c.*	*Per cent*		
Rape pasture	[a] 529	5.44	16.5	3.71	7.5–8.5	None.
Bluegrass pasture	[a] 520	3.3	10.0	2.90	7.6	Do.
Alfalfa hay, 2 pounds; oats, 1.5 pounds	[a] 183	3.91	22.3	1.10	7.8–8.6	Few.
Alfalfa hay, 2 pounds; corn, 1 pound	[b] 275	4.38	17.8	1.48	7.8–8.2	Do.
Bluegrass pasture; mixture of 3 parts oats and 1 part corn, 1.5 pounds	[a] 330	3.91	11.0	1.52	7.5–7.7	None.
Bluegrass; mixture of 2 parts corn, 2 parts oats, 1 part bran, 1.5 pounds	[a] 262	4.5	7.4	2.1	7.6–8.0	Few.
Alfalfa hay, 2 pounds; mixture of 2 parts corn, 2 parts oats, and 1 part bran, 1.5 pounds	[a] 290	4.61	29.0	1.45	8.3	Many.
Alfalfa hay, 2 pounds; bluegrass; mixture of 3 parts oats and 1 part corn, 1.5 pounds	[a] 350	4.51	29.3	1.69	8.1–8.8	Few.
Corn silage, 2.5 pounds; mixture of 1 part corn and 3 parts oats; 1 pound	[b] 118	4.30	0.10	1.69	7.3	None.
Corn silage, 2.5 pounds; mixture of 3 parts oats and 1 part bran, 1.75 pounds	[b] 178	6.96	0.9	1.01	6.8–8.0	Many.
Corn silage, 1.2 pounds; clover hay, 1.6 pounds	[b] 320	4.1	27.4	1.90	8.5	Solids, not crystals.
Rape pasture; mixture of 3 parts oats and 1 part corn, 2 pounds	[a] 272	5.590	2.72	2.39	8.3	Many.
Rape and bluegrass pasture; mixture of 3 parts corn and 1 part oats, 0.5 pound	[b] 410	5.41	2.72	2.54	7.8–8.6	Few.

[a] In 12 hours. [b] In 24 hours.

RAPE V. BLUEGRASS PASTURE

Since rape pasture is often provided for sheep, this feed was used for study in contrast with bluegrass pasture, which is usually rated well whenever it can be obtained. It was found that the sheep fed rape produced somewhat more basic urine than those fed bluegrass. Urine from the former contained 5.44 per cent total solids, and 16.5 c. c. of 0.20 N HCl were required to neutralize the ash; whereas the urine from sheep fed bluegrass contained 3.3 per cent total solids, and 10.0 c. c. of HCl were required to neutralize the ash.

This difference might not seem great enough to be significant, but it was noticed that when 2 pounds of grain were fed the rape urine ash was neutralized and the urine deposited crystals upon standing for several days. This indicates that the rape contained more calcium, magnesium, phosphate, and silicate compounds than the bluegrass. The latter gave no crystal deposits when the urine stood in a flask for a month, and the addition of grain to the ration did not yield a neutral urine ash. On the contrary 11.0 c. c. of 0.20 N HCl per 100 c. c. of urine were required to neutralize it. This alkalinity was due largely to the production of potassium carbonate in the formation of the ash material.

Alfalfa—Oat Ration

When grain was fed with alfalfa hay in the proportion of 2 pounds of hay to 1.5 pounds of oats, 183 c. c. of urine per sheep were produced in 12 hours. This urine had an average pH value of 8.2, contained 3.91 per cent of solids and 1.10 per cent of ash. In this case the calcium, potassium, and other bases of the alfalfa were more nearly neutralized by the phosphoric acid from the oats, and only 22.3 c. c. of 0.20 N HCl were required to neutralize the ash from a 100 c. c. sample. Two other sheep on the same ration produced 310 c. c. of urine each, which had the same pH value and contained 3.7 per cent of solids and 1.26 per cent of ash. The urine ash was less basic than before, and only 16.4 c. c. of 0.20 N HCl were required for the ash from a 100 c. c. sample.

The low percentage of solids (3.7 per cent) in the urine would indicate an efficient utilization of the food nutrients; besides, the alkalinity was not high enough to be irritating.

Oats, Corn, and Bluegrass Pasture Ration

When bluegrass pasture was substituted for the alfalfa hay and 1.5 pounds of grain (consisting of 3 parts oats and 1 part corn) were fed, the large quantity of potassium (K_2O), common to most pastures, averaged nearly half of the total ash content, yet gave the urine ash a less basic reaction than alfalfa and oats. The urine output was 330 c. c. per sheep in 24 hours, contained 3.91 per cent total solids, 1.52 per cent ash, and 11.0 c. c. of 0.20 N HCl were required to neutralize the ash from 100 c. c. of urine.

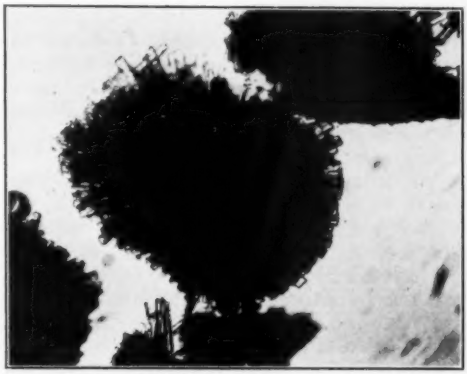

Figure 4.—Crystals obtained from sheep urine that had stood for 24 hours

Since the ash was quite water soluble, it was hardly to be expected that calculi would be formed on a grain and bluegrass pasture ration. Reports from experienced sheepmen indicate that calculi rarely, if ever, form when bluegrass pasture constitutes an appreciable part of the ration.

Alfalfa, Grain, and Bran Ration

When wheat bran is introduced into a ration it carries with it a large mineral content, especially phosphorus compounds. These readily form water-insoluble combinations with calcium, magnesium, and aluminum, and gravel is deposited in the kidney and bladder, thereby often causing serious trouble to male sheep. The gravel tends to combine to form a conglomerate either in the kidneys, the ureters, the bladder, or the urethra, which makes it impossible for the urine to be voided. When bran is fed with alfalfa hay, deposits of calcium phosphate, ammonium oxalate, and aluminum phosphate,

etc., form in the urine a few hours after it has been passed. A photograph of crystals obtained from urine which had stood for 24 hours is shown in Figure 4.

These crystals often have a definite structure and are quite easily distinguished from the organic solids always present in urine. The crystals are heavier and readily settle to the bottom of the flask, whereas the organic débris stays in suspension longer, thus making it possible to obtain an estimate of the amount of each. When sheep are on pasture there is no evidence of such solids in the urine, but upon the addition of grain, especially wheat bran, even to a pasture ration, there will be a tendency for gravel to form when the urine stands a day or more. This was noticed especially in the case of sheep on rape pasture. As long as the sheep were kept on rape no crystals appeared in the urine, but when corn and oats were given in addition, gravel formation was noticed in all samples. About half of the deposit was mineral material, chiefly calcium phosphate and aluminum silicate. This study of the relation between the urinary ash of sheep and different feeds is being continued for the purpose of finding a ration that will be most effective in preventing calculi formation.

Silage and Grain Ration

When corn silage was fed with a grain mixture of corn and oats, the urine ash was nearly neutral. When alfalfa or clover hay was fed with silage and grain, the urine ash was more alkaline than when silage and grain were fed. When the ration consisted of mangels or alfalfa hay (Table 1) the urine ash was strongly alkaline. Mangels have an effect on the reaction of the urine ash just the opposite to that of silage.

The reaction of the urine is further affected by the rate at which urease enzymes convert urea to ammonium salts. Williard [4] has shown that the rate of crystal growth and the size of the crystals formed are affected by the rate of the urease reaction. When urea was used for this purpose instead of ammonium hydroxide, the precipitation was granular. The authors believe that their results warrant the hypothesis that the retention of urine in the bladder is conducive to the formation and growth of crystals. When the ration fed is composed of feeds which produce insoluble mineral compounds and is lacking in succulence, and when the atmospheric temperature is low, the urine becomes very concentrated and is likely to be retained longer in the bladder. This gives urease sufficient time to form ammonium salts which in turn increases the alkalinity of the urine to a point where certain insoluble compounds are precipitated. The crystals may become large enough to close the urinary tract.

SUMMARY

The replies received from a questionnaire sent to sheepmen indicate that the occurrence of calculi in sheep is fairly general in the United States, but is more frequent in some sections than in others.

Twelve positive cases of calculi were observed and studied by the writers, and 25 autopsies were performed. Post-mortem data on 219 lambs were obtained. Ten of these lambs had calculi.

Calculi were found in the kidneys, the ureters, the bladder, and the urethra, but in only a few cases were they found in all of these parts in the same sheep.

[4] Willard, H. H. Information to the authors.

The calculi obtained and analyzed were composed largely of calcium phosphate and organic matter. They varied widely in size and in degree of hardness.

Calculi were found more often in the processus urethrae (worm) than in any other part of the urinary tract. The S-curvature of the urethra in male sheep offers a mechanical difficulty which frequently prevents the passage of calculi beyond this point. This is the place where large calculi are most likely to lodge and cause complete stoppage.

Partial or complete inability to urinate is the most positive symptom of calculi formation. When calculi are present the wool around the end of the sheath is invariably wet. In severe cases, the back becomes arched because of the great effort required to urinate.

If the calculus is in the worm, and the worm is removed, recovery is rapid. The writers do not know of a successful method of dislodging large calculi from the S-curvature of the urethra.

It was found that one attack was likely to be followed by a second or a third attack. In no instance did the animal survive the third attack.

Experiments were made to determine how different rations would affect the reaction or composition of urine. These experiments were conducted largely with wethers and ewes 1 year old and older, and covered a period of over two years. Forty-two different sheep were used and 104 samples of urine were collected.

When roots were fed, 2,200 to 2,500 c. c. of urine per sheep were produced in 24 hours, whereas from sheep on dry feed the average production was only 200 to 250 c. c., but pasture increased this amount to about 600 c. c. per half day.

The reaction of the fresh urine varied from pH 6.8 to 9.0, depending on the feed of the animal. Sheep on alfalfa or beet rations produced a very basic urine ash, but when a cereal grain or bran was added to the ration the reaction of the urine was nearly neutral. The presence of the enzyme urease in urine also is responsible for it becoming basic rapidly upon standing due to the formation of ammonium carbonate from urea. This accounts for part of the large deposits of organic residues formed as the enzyme action continues making the urine much more basic.

When the ration was utilized to best advantage by the sheep, about 3 per cent of solids were found in the urine. On the most undesirable rations the sheep produced a urine containing 6 to 8 per cent of solids, which indicates a large waste of nutrient material. Moreover, the urine ash was highly basic. It seems probable that such urine would irritate the urinary tract and thus account for the observed cases of marked inflammation of the urethra in the absence of calculi. When sheep were fed alfalfa or clover hay or roots alone a highly basic urine was produced.

Certain mineral compounds are sometimes precipitated from the urine and the crystals cluster around an organic nucleus, which results in the formation of calculi.

The nature of the urine affords an important guide to the utilization of a feed and has a notable effect on the production of calculi.

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